PROCEEDINGS

OF THE

INDIAN ACADEMY OF SCIENCES

VOL. XXXVI

SECTION B

BANGALORE CITY
PRINTED AT THE BANGALORE PRESS, MYSORE ROAD
1953

Q
73
.I36
Sect. B
v. 36

CONTENTS

SECTION B—VOL. XXXVI

PAGE

No. 1—July 1952

Embryological Studies in the Leguminosæ. IV. A Contribution to the Embryology of *Neptunia triquetra* Benth. . . . V. R. Dnyansagar 1

Technique of Mass Multiplication of *Stenobracon deesæ* (Cam.) Hymenoptera: Braconidæ for Use Against Sugarcane and Maize Borers . . . . . E. S. Narayanan and T. V. Venkatraman 12

Soil Conditions and Root Diseases: IV—The Effect of Micro-Elements on the Occurrence of Bacteria, Actinomycetes and Fungi in Soils . . . . . (Miss) C. B. Sulochana 19

Embryology of *Anagallis pumila* Swartz. . . . . . M. V. S. Raju 34

No. 2—August 1952

Fungi Imperfecti from Madras—I . . . . . C. V. Subramanian 43

Soil Conditions and Root Diseases. V. Symptomatology of Wilted Cotton and Red Gram . . . G. Satyanarayana and R. Kalyanasundaram 54

Studies in the Order Parietales. III. Vascular Anatomy of the Flower of *Carica papaya* L., with Special Reference to the Structure of the Gynæceum . . . . . Shanti Devi 59

The Amylose and the Amylopectin Contents of Rice and Their Influence on the Cooking Quality of the Cereal . . . . . B. Sanjiva Rao, A. R. Vasudeva Murthy and R. S. Subrahmanya 70

Fertilization Value of Tank Silts. II. Phosphorus and Reproductive Vigour of *Oryza sativa* L. . . C. S. Venkata Ram and K. V. Srinivasa Pai 81

Additions to the Fungi of Madras—XIII . . . . . T. S. Ramakrishnan, K. V. Srinivasan and N. V. Sundaram 85

No. 3—September 1952

Observations on Ergots on *Pennisetum* and Other Grasses . . . . . T. S. Ramakrishnan 97

Ascorbic Acid and *Fusarium* Wilted Plants . . . R. Kalyanasundaram 102

Observations on the Food and Feeding of the Indian Mackerel, *Rastrelliger canagurta* (Cuvier) . . . . . B. S. Bhimachar and P. C. George 105

Decomposition of Paddy and Bajra *Pennisetum typhoideum* Straws by Fungi Commonly Found in Allahabad Soils . Ravi Prakash and R. K. Saksena 119

***Glomerella psidii* (Del.) Sheld. and *Pestalotia psidii* Pat. Associated with a Cankerous Disease of Guava** . . . . . N. S. Venkatakrishniah 129

PAGE

No. 4—October 1952

Spores and Pollen from Tertiary Lignites from Dandot, West Punjab (Pakistan) . . . . . K. P. Vimal 135

Oxygen Consumption and Viability of *Chanos chanos* (Forskål) in Relation to Size . . . . . R. Viswanathan and P. R. S. Tampi 148

Fungi Imperfecti from Madras—II . . . . . C. V. Subramanian 160

A New Species of Cœlomic Trematode of the Genus *Staphylorchis* from the Tiger Shark *Galeocerdo tigrinus* from Indian Waters . . Mary Samuel 169

No. 5—November 1952

Relative Value of Nitrogen and Phosphate in Some Organic Manures and Inorganic Fertilisers and Their Combinations as Judged by Their Recovery by Crops . . . . . S. V. Desai and B. V. Subbiah 181

A Contribution to the Embryology of *Chlorophytum attenuatum* Bak. . . . . . K. Subramanyam and A. Nagaraja Rao 191

Studies in the Physiology of Phanerogamic Parasitism with Special Reference to *Striga lutea* Lour. and *S. densiflora* Benth. on *Andropogon sorghum* Hack. II. The Influence of Temperature and Moisture on the Germination and Growth of *S. lutea* and *S. densiflora* . . . . S. Solomon 198

An Ectoparasite on the Larvæ of *Anthrenus vorax* Waterhouse . . . . . P. K. Ayyappa and P. S. Cheema 215

No. 6—December 1952

Fungi Imperfecti from Madras—III. *Beltraniella* gen. nov. . . . . . C. V. Subramanian 223

Soil Conditions and Root Diseases. VI. Germination of Conidia of *Fusarium vasinfectum* in Micro-Element Amended Soils . Miss C. B. Sulochana 229

Soil Conditions and Root Di eases. VII. Response of Cotton Plants to Micro-Element Amendments and Its Relation to Disease Development . . . . . Miss C. B. Sulochana 234

Morphological and Other Studies on Two *Nocardia* Species . . . . . J. R. Vakil, S. R. Khambata and J. V. Bhat 243

Embryology of the Dipsacaceæ. I. Glands, the Male and Female Gametophytes . . . . . B. A. Razi and K. Subramanyam 249

EMBRYOLOGICAL STUDIES IN THE LEGUMINOSÆ

IV. A Contribution to the Embryology of *Neptunia triquetra* Benth.

By V. R. Dnyansagar, M.Sc.

(Department of Botany, College of Science, Raipur, M.P.)

Received January 12, 1952

(Communicated by Prof. L. Narayan Rao, F.A.SC.)

The present paper deals with the embryology of *Neptunia triquetra*, a member of the Mimosaceæ. The author has already studied the embryology of three Mimosaceous plants, *Leucæna glauca* (Dnyansagar, 1949), *Mimosa hamata* (Dnyansagar, 1951) and *Pithecolobium saman* (Dnyansagar, 1951) and the present paper is the fourth in the series. *Neptunia triquetra* shows a number of characters similar to those of the above three species, these being a single layered tapetum of the secretory type, a massive nucellus, extensive parietal tissue in the ovule, two integuments, of which the inner develops first and the outer overgrows it, hooked synergids with filiform apparatus, the division of the primary endosperm nucleus before the first division of the oospore and a suspensorless massive proembryo. Further, this species resembles *Leucæna glauca* in simple pollen-grains and *Mimosa hamata* and *Pithecolobium saman* in hypodermal origin of the archesporium.

Material and Methods

The material was collected from the garden of the College of Science, Nagpur. It was fixed in formalin-acetic-alcohol and Randolph's modification of Navaschin's fluid (Johansen, 1940). Sections cut at a thickness of 8–15 μ were stained in iron-alum hæmatoxylin. For pollen-preparations, aniline oil-gentian violet (Wodehouse, 1935) and methyl green-glycerine jelly (Wodehouse, 1935) were used.

The Inflorescence and the Flower

Neptunia triquetra is a low prostrate perennial herb with slender ascending compressed stems. The inflorescence is an axillary globose head. It consists of 15–20 flowers which are generally of three types, the lower ones being sterile, middle ones being male and the uppermost being bisexual. The lower flowers serve to attract insects for pollination. There is thus the division of labour as in the inflorescence of *Neptunia oleracea* (Geitler, 1927). It is only rarely that all the flowers are found to be bisexual. The

total number of fruits formed per inflorescence is 2–5. Most of the inflore scences are attacked by red ants when they are just beginning to put forth fruits with the result that only a few ripe fruits are formed per inflorescence.

The floral parts arise in acropetal succession and are cyclic in their arrangement as in *Acacia Baileyana* (Newman, 1933), *Leucæna glauca* (Dnyansagar, 1949), *Mimosa hamata* (Dnyansagar, 1951) and *Pithecolobium saman* (Dnyansagar, 1951). The formula is K (5), C_5, A10, G1.

MICROSPOROGENESIS

The archesporial cells are hypodermal in origin. They are, however, usually distinguished only when one or two layers of cells have been formed as in *Albizzia lebbek* (Maheshwari, 1931), *Neptunia oleracea* (Singh and Shivapuri, 1935) and *Leucæna glauca* (Dnyansagar, 1949).

The wall of the young anther consists of four layers of cells internal to the epidermis (Fig. 1). The outermost is the endothecium whose cells acquire fibrous thickenings at maturity (Fig. 2). Next in order, are the usual two middle layers and a single layered tapetum. The latter is of the secre-tory type and its cells remain uninucleate throughout as in all the investigated species of the Mimosaceæ. In the mature anther, only the endothecial layer of the parietal tissue persists while the rest of the layers become used up during sporogenesis.

About 16–32 microspore mother-cells are produced per microsporangium and are at first closely packed together (Fig. 1). They are polygonal in outline and possess densely staining cytoplasm (Fig. 1). Afterwards, these separate from one another, become rounded and each secretes a thick wall as in *Leucæna glauca* (Dnyansagar, 1949).

The usual two reduction divisions then follow. Cytokinesis takes place by furrowing and tetrahedral tetrads of microspores are produced (Fig. 2) as in *Neptunia oleracea* (Singh and Shivapuri, 1935) and *Leucæna glauca* (Dnyansagar, 1949).

The uninucleate pollen-grains separate from one another as in *Neptunia oleracea* (Singh and Shivapuri, 1935) and *Leucæna glauca* (Dnyansagar, 1949) and there is never any formation of compound grains. This feature is indeed interesting since, generally, the pollen-grains of the Mimosaceæ are of con-globate type (Wodehouse, 1935). According to Wodehouse (1935), the formation of simple grains in the Mimosaceæ is a primitive character. This may apply to the present plant also.

Neptunia triquetra was formerly placed under the genus *Mimosa* as *Mimosa triquetra* Vahl. (Hooker, 1879). Bentham (Hooker, 1879) assigned

it to the genus *Neptunia*. The nature of the pollen-grains as revealed from the present study, supports the inclusion of this species in the genus *Neptunia*. The pollen-grains in the genus *Mimosa* are compound and, as far as known, they are never of the simple type. In the present plant, the pollen-grains are simple and resemble those found in *Neptunia oleracea* (Singh and Shivapuri, 1935) so that this species should better be placed under *Neptunia* rather than under *Mimosa*. Further, according to Wodehouse (1935), a compound pollen-grain represents an advanced condition over that of a simple grain. This suggests that the genus *Mimosa* may be comparatively more highly evolved than *Neptunia*.

The mature pollen-grain is variously shaped but the commonest form is triangular or spherical (Fig. 3). It is about 45–50 μ in diameter. It is of the *Prosopis glandulosa* type (Wodehouse, 1935). Similar type of the grain has been described in *Neptunia oleracea* (Singh and Shivapuri, 1935) and *Leucæna glauca* (Dnyansagar, 1949).

Each cell has vacuolated cytoplasm and is uninucleate when young. It becomes bi-celled at the time of its shedding.

Chromosome Number

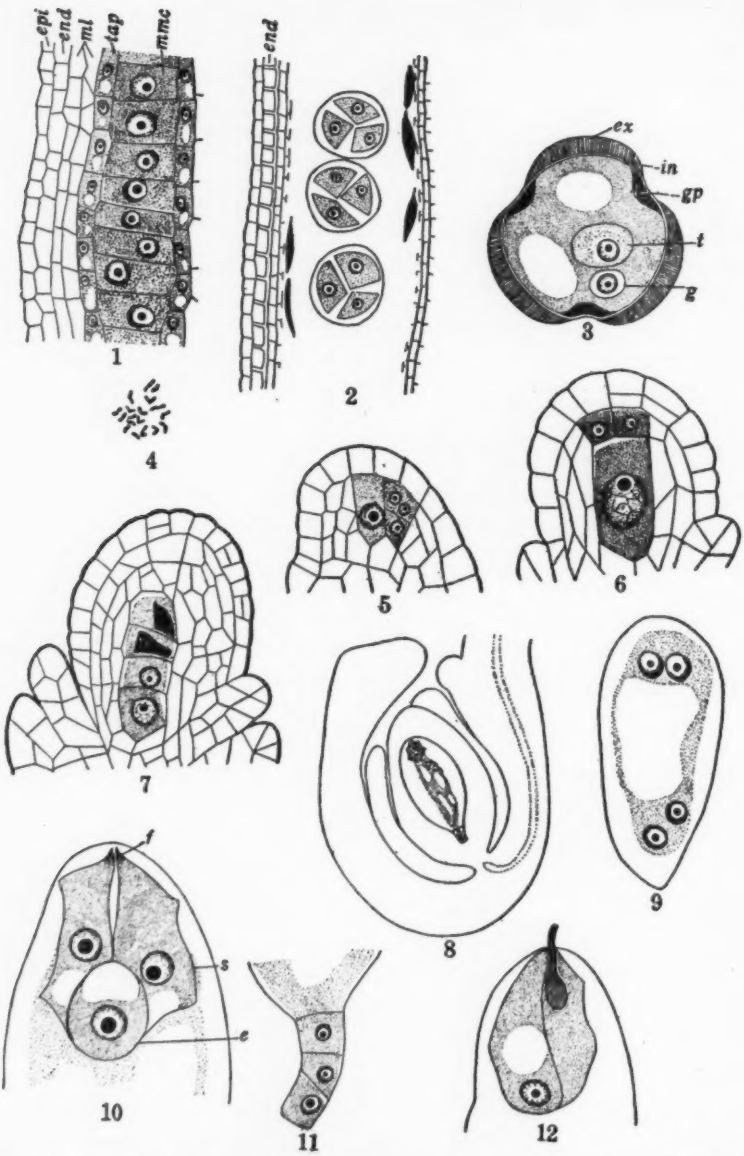
Chromosome counts were made from polar views of the metaphase in the first reduction division of the microspore mother-cell. The *n* chromosome number determined in this species is 18 (Fig. 4).

Megasporogenesis

The ovary bears 2–8 ovules in two alternating rows on its ventral suture. Their gradual assumption of anatropous form after acute curvature upwards is same as in *Albizzia lebbek* (Maheshwari, 1931), *Neptunia oleracea* (Singh and Shivapuri, 1935), *Leucæna glauca* (Dnyansagar, 1949), *Mimosa hamata* (Dnyansagar, 1951), *Pithecolobium saman* (Dnyansagar, 1951) and several other Leguminous species.

The nucellus is here massive from the very beginning like that in the previously investigated species of the Mimosaceæ, of which it seems to be a characteristic feature.

Epidermal cells at the micropylar end form a cap (Fig. 18) by repeated divisions and thus add to the increase of the original parietal tissue formed by the activity of the primary parietal cell. According to Guignard (1881), formation of such an epidermal cap is a common feature of the Leguminosæ. Recently, Pantulu (1945) has observed the cap in a number of species of the Cæsalpiniaceæ. Among the Mimosaceæ, it has been reported in *Acacia*



FIGS. 1–12. Fig. 1. Longitudinal section of part of anther showing microspore mother-cells, *mmc*, tapetal layer of uninucleate cells, *tap*, middle layers, *ml*, endothecium, *end.*, and epidermis, *epi* × 360. Fig. 2. Longitudinal section of part of anther showing tetrahedral tetrads of microspores, remnants of disorganised tapetum and inner middle layer,

outer middle layer, endothecium, *end*, with its inner and radial walls thickened and epidermis, × 225. Fig. 3. Mature pollen-grain with exine *ex*, intine, *in*, 3 germ pores, *gp*, tube-cell, *t* and generative cell, *g*. × 600. Fig. 4. Polar view of metaphase in first meiotic division showing 18 chromosomes, × 1,900. Fig. 5. Longitudinal section of nucellus showing two archesporial cells. The cell on the right side has divided into two cells and the parietal cell thus formed is dividing anticlinally, × 500. Fig. 6. Longitudinal section of nucellus showing the megaspore mother-cell and the parietal layer of two cells. The epidermal cells have also divided periclinally, × 500. Fig. 7. Longitudinal section of ovule showing a deep-seated linear tetrad of megaspores, of which two are degenerating and primordia of two integuments, × 500. Fig. 8. Longitudinal section of ovule at the mature embryo-sac stage, showing formation of micropyle by the outer integument alone, × 180. Fig. 9. Four-nucleate stage of embryo-sac, × 500. Fig. 10. Micropylar part of embryo-sac showing egg-apparatus; egg, *e*. synergids, *s*, with filiform apparatus, *f*. × 600. Fig. 11. Chalazal part of embryo-sac showing three antipodal cells, × 600. Fig. 12. Entry of pollen-tube into a synergid, × 500.

Baileyana (Newman, 1933–34), *Neptunia oleracea* (Singh and Shivapuri, 1935) and has now been seen by the author in *Leucæna glauca*, *Mimosa hamata*, *Pithecolobium saman* and the present plant.

The primordia of integuments appear after the differentiation of the archesporium. The inner integument develops first and the outer one follows it immediately. Each integument to begin with is bi-layered (Fig. 7). The inner integument remains bi-layered throughout and it is only at the top when the embryo-sac becomes mature that it usually becomes tri-layered. The outer integument, however, becomes several layered at the top and 3-4 layered below by the time the sac has attained maturity.

The micropyle (Fig. 8) is peculiar in two ways. In the first instance, it is formed by the outer integument alone, the inner integument not extending beyond the nucellus and being perfectly at level with it. Another peculiarity of the micropyle is due to the right side of the outer integument that is the one near the funiculus developing for a very short length beyond the nucellus and the left side that is the one away from the funiculus curving and developing at right angles to the longitudinal axis of the nucellus. It recalls the "zigzag" micropyle as described by Pantulu (1945) in *Cassia* with just a little difference that while in *Cassia*, the inner integument develops a little beyond the nucellus and forms the lower part of the micropyle, n the present plant, the inner integument does not at all contribute to its ormation.

The archesporium differentiates in the hypodermal region of the nucellus usually as a single cell before the appearance of the primordia of integuments and divides, as usual, by a periclinal wall to form on the outside a parietal cell (Fig. 6); this by repeated periclinal and anticlinal divisions forms the

extensive parietal tissue which seems to be a characteristic feature of the Cæsalpiniaceæ and the Mimosacæ.

In one case, two archesporial cells in contact with each other were found to be differentiated (Fig. 5). An archesporium consisting of more than one cell has been reported in *Albizzia lebbek* (Maheshwari, 1931), *Neptunia oleracea* (Singh and Shivapuri, 1935), *Cassia* (Pantulu, 1945) and *Acacia farnesiana* (Narasimhachar, 1948). One of the two archesporial cells in the above case has divided by a periclinal wall and the parietal cell thus formed was dividing anticlinally (Fig. 5). Whether both of these archesporial cells eventually functioned to give rise to two embryo-sacs could not be determined.

As a consequence to the external parietal tissue referred to above, the megaspore mother-cell becomes deep seated. Further, it gives rise to a linear tetrad of megaspores (Fig. 7) after two reduction divisions. The chalazal megaspore forms the embryo-sac which is of the normal 8-nucleated type. It tapers towards the chalazal end (Fig. 9). The egg-apparatus consists of two hooked synergids with filiform apparatus and the egg which protrudes below (Fig. 10). Dahlgren reviewed the literature on hooked synergids in 1938. Since then, they have been reported in several species of the Leguminosæ. The author listed them in his third paper (Dnyansagar, 1951). The present case is an addition to the list.

The antipodals are definite cells (Fig. 11) as in the previously investigated species of the Leguminosæ and were found to be persistent upto the first division of the primary endosperm nucleus.

The synergids are also found to persist for a long time. They are intact even at the 3-celled stage of the proembryo (Fig. 14) when several endosperm nuclei have been formed. At the 4-celled stage of the proembryo, they undergo disorganisation but are still recognisable (Fig. 15). Such persistence has been observed in one case by the author in *Pithecolobium saman* (1951), where the remnants of the synergids are found at the 4-celled stage of the proembryo.

The starch-grains appear in the embryo-sac at the 8-nucleated stage. Dahlgren (1939) who has reviewed the literature on starch-grains in the embryo-sacs, states that the maximum production of starch-grains seems to take place just before fertilisation. The author's observation in *Mimosa hamata* (1951), *Pithecolobium saman* (1951) and in the present case agrees with his findings.

Germination of the Pollen-Grains

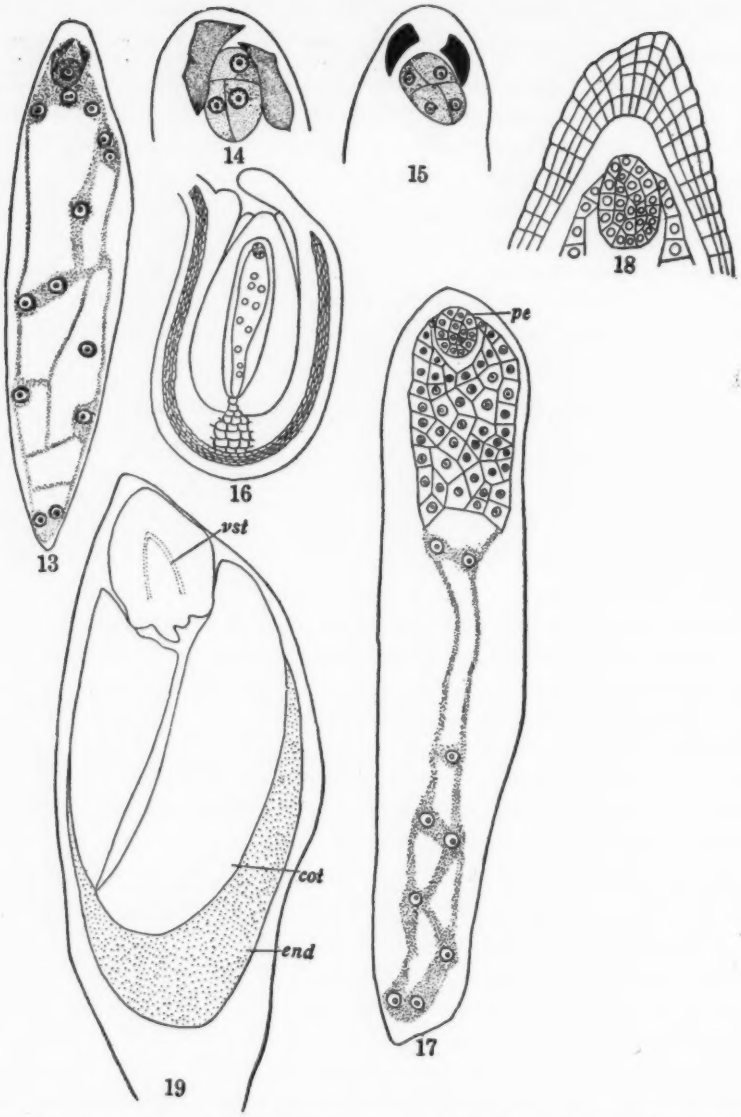
Pollen-grains were germinated on 10%, 15%, 20%, 25% and 30% cane sugar solutions to which neutral red was added for vital staining. 30% to 40% pollen-grains germinated after about 2 hours in solution from 20% concentration onwards and sent out tubes. The generative nucleus was observed to move first in the tube. More or less similar results were obtained by using 10% agar and 15%, 20%, 25% and 30% cane sugar solutions. The tube nucleus subsequently disorganised. A large number of styles were dissected after macerating in 40% acetic acid at 45° C. Bi-celled grains were found to be lodged in the hollow of the stigma. In some, the tubes without any nuclei were seen while in others, tubes with distinct generative tube nuclei were formed.

Fertilisation, Endosperm and Embryo

Details of fertilisation could not be observed. In one case, however, a pollen-tube was seen in one of the synergids (Fig. 12). This suggests that the passage of the pollen-tube to the egg is through a synergid. Such a case has been observed by Warmke (1943) in *Taraxacum* and in *Oxalis* by Krupko (1944).

The primary endosperm nucleus undergoes many divisions to give rise to a number of free nuclei before the first division of the oospore (Fig. 13) as in *Acacia farnesiana* (Narasimhachar, 1948), *Leucæna glauca* (Dnyansagar, 1949), *Mimosa hamata* (Dnyansagar, 1951) and *Pithecolobium saman* (Dnyansagar, 1951). Wall formation takes place first in the micropylar region (Fig. 17) and then proceeds towards the chalazal end of the sac. There is no trace of the endosperm left in the mature seed as it is resorbed completely by the developing embryo.

The first division of the oospore is transverse (Fig. 14) as in *Acacia Baileyana* (Newman, 1934), *Acacia farnesiana* (Narasimhachar, 1948), *Leucæna glauca* (Dnyansagar, 1949), *Mimosa hamata* (Dnyansagar, 1951) and *Pithecolobium saman* (Dnyansagar, 1951). The terminal and the basal cells then divide by a longitudinal wall (Figs. 14 and 15), the former being the first to divide. After the 4-celled stage, the development of the proembryo is rather irregular. It becomes spherical and develops no suspensor (Fig. 17). Figure 19 shows a very advanced stage of the embryo showing two cotyledons, a plumule and a radicle with vascular strands in these organs. According to Johansen (1950), there are two types of embryogeny in the Mimosaceæ, one with and the other lacking a suspensor. A massive type of the proembryo without a suspensor found in the present plant has been described



FIGS. 13–19. Fig. 13. Longitudinal section of ovule showing free endosperm nuclei, undivided oospore and two persistent synergids, × 225. Fig. 14. 3–Celled proembryo and two persistent synergids, × 500. Fig. 15. 4–Celled proembryo and remnants of synergids, × 500. Fig. 16. Longitudinal section of ovule at the 3-celled proembryo stage, showing vascular strand in the outer integument, × 180. Fig. 17. Longitudinal section of

ovule showing massive proembryo, *pe* and formation of cellular endosperm in micropylar region, × 112. Fig. 18. Longitudinal section of part of ovule showing the epidermal cap, several celled spherical proembryo and part of cellular endosperm, × 225. Fig. 19 Longitudinal section of seed showing advanced stage of the embryo with two cotyledons, *cot*, still partially surrounded by the unabsorbed endosperm, *end*, vascular strand, *vst*, have been differentiated, × 25.

in several species of the Mimosaceæ, *e.g.*, *Acacia Baileyana* (Newman, 1934), *Acacia farnesiana* (Narasimhachar, 1948), *Leucæna glauca* (Dnyansagar, 1949), *Mimosa hamata* (Dnyansagar, 1951) and *Pithecolobium saman* (Dnyansagar, 1951). Johansen (1950) has stated *Cercis siliquastrum* as the example of a species whose embryo develops a suspensor. According to his classification of the embryo, development of the embryo in *Acacia Baileyana* follows "Trifolium Variation, Onagrad type, save that a suspensor is not formed". The embryogeny in the present plant is similar in the essential respects to that of *Acacia Baileyana* and may therefore, be assigned to the same category.

Prior to the division of the oospore, there is a vascular strand only in the region of the chalaza (Fig. 8). But when the division of the oospore takes place, strands of elongated cells begin to appear in the outer integument and reach even as far as the micropyle. These strands are organised into prominent vascular elements (Fig. 16). The author has now observed such integumentary vascular strands in *Leucæna glauca*, *Mimosa hamata* and *Pithecolobium saman* during first fertilisation stages. Such a condition has been described by Nitolitzky (1926) in a few Papilionaceæ and by Rau (1950) in *Cassia tora*. The author agrees with the view of Rau (1950) that this development of the integumentary strands may be due to enormous increase in the size of the ovule.

Summary

1. *Neptunia triquetra* Benth. is a low prostrate perennial herb with slender ascending compressed stems.

2. The inflorescence is an axillary globose head and consists of 15–20 flowers. The total number of fruits formed per inflorescence is 2–5.

3. The primary archesporium is usually differentiated late. The tapetum is of the secretory type. It is unilayered and its cells remain uninucleate throughout.

4. The microspore mother-cells first become separate from one another and then form tetrahedral tetrads of microspores after undergoing usual reduction divisions.

. The pollen-grains are simple and are of the *Prosopis glandulosa* type. At the time of shedding, they are bi-celled.

6. The *n* chromosome number is 18.

7. The ovules are anatropous and have two integuments. The micropyle is formed by the outer integument alone and is at right angles to the longitudinal axis of the nucellus.

8. Megasporogenesis follows the normal course. The archesporium is hypodermal and usually single celled. An epidermal cap is formed over the original parietal tissue. The latter becomes extensive as a result of the activity of the primary parietal cell. A linear tetrad of megaspores is formed, of which the chalazal functions.

9. The mature embryo-sac is of the normal 8-nucleated type. The synergids are hooked and each has a filiform apparatus. They are persistent even when the primary endosperm nucleus has formed several free nuclei. The antipodals form definite cells and persist as late as the first division of the primary endosperm nucleus. Starch-grains appear in the mature sac.

10. The primary endosperm nucleus divides several times before the first division of the fertilised egg. Wall formation in the endosperm commences from the micropylar end.

11. The development of the embryo follows the Trifolium Variation Onagrad type, except that a suspensor is not formed.

12. There is a vascular strand only in the chalazal region of the ovule prior to the fertilization. During post-fertilisation stages, however, the strand reaches upto the micropyle through the outer integument.

In conclusion, the author wishes to express his gratitude to Prof. R. L. Nirula, under whose guidance the work was carried out for helpful suggestions and criticism throughout the course of this investigation.

LITERATURE CITED

Dahlgren, K. V. O. .. "Hakenbildungen bei Synergiden," *Zweite Mitteilung. Svensk Bot. Tid.*, 1938, **52**, 221–37.

———— .. "Sur la présenee d' amidon dans le sac embryonnaire ches les Angiospermes II," *Sar. ur. Bot.*, 1939, 221–31.

Dnyansagar, V. R. .. "Embryological studies in the Leguminosæ—I. A contribution to the embryology of *Leucæna glauca* Benth.," *Jour. Ind. Bot. Soc.*, 1949, **28**, 97–107.

———— .. "Embryological studies in the Leguminosæ—II. A contribution to the embryology of *Mimosa hamata*," *Ibid.*, 1951, **30**, 100–107.

Dnyansagar, V. R. .. "Embryological studies in the Leguminosæ—III. A contribution to the embryology of *Pithecolobium saman* Benth. syn. *Enterolobium saman* Prain," *Proc. Ind. Acad. Sci.*, 1951, **34 B**, 188–98.

Engler, A. and Prantl, K. .. "Die Naturilichen Pflanzenfamilien," *Zweite Auflaze*, 1926.

Geitler, L. .. "Zur Morpholgie der Infloreszenzen und Bluten von *Neptunia oleracea*," *Ber. Deutsch. Bot. Ges.*, 1927, **45**, 36–39 (quoted from Singh and Shivapuri, 1935).

Guignard, L. .. "Recherches d' embryogénie Végétale Comparée I. Legumineuses," *Ann. Sci. Nat. Bot.*, 1881, **12**, 5–166.

Hooker, J. D. .. *The Flora of British India*, Kent, 1879.

Johansen, D. A. .. *Plant Microtechnique*, New York, 1940.

———— .. *Plant Embryology*, Waltham, Mass., 1950.

Krupko, S. .. "On the sterility of *Oxalis cernua* in the Mediterranean," *Przyroda*, 1944, 1–32.

Maheshwari, P. .. "Contribution to the morphology of *Albizzia lebbek*," *Jour. Ind. Bot. Soc.*, 1931, **10**, 241–64.

Narasimhachar, S. G. .. "A contribution to the embryology of *Acacia farnesiana* L. (Willd.)," *Proc. Ind. Acad. Sci.*, 1948, **28**, 144–49.

Newman, I. V. .. "The life-history of *Acacia Baileyana* (F.V.M.)," *Jour. Linn. Soc.*, 1933, **49**, 145–67.

———— .. "Studies in the Australian Acacias—III. Supplementary observations on the habit, carpel, spore production and chromosomes of *Acacia Baileyana* (F.V.M.)," *Proc. Linn. Soc.*, New South Wales, 1934, **59**, 237–50.

———— .. "Studies in the Australian Acacias—IV. The life-history of *Acacia Baileyana* F.V.M. Part 2. Gametophytes, fertilisation, seed production, germination and general conclusion," *ibid.*, 1934, **59**, 277–313.

Pantulu, J. V. .. "Studies in the Cæsalpiniaceæ—I. A contribution to the embryology of the genus *Cassia*," *Jour. Ind. Bot. Soc.*, 1945, **24**, 10–24.

Rau, Anantswamy, M. .. "Integumentary vascular tissue in *Cassia tora* Linn.," *Curr. Sci.*, 1950, **19**, 186–87.

Singh, B. and Shivapuri, T. N. "The gametophytes of *Neptunia oleracea* Lour.," *Proc. Ind. Acad. Sci.*, 1935, **1**, 423–34.

Warmke, H. E. .. "Macrosporogenesis, fertilisation and early embryology of *Taraxacum Koksaghys*," *Bull. Torrey. Bot. Club.*, 1943, **70**, 164–73.

Wodehouse, R. P. .. *Pollen Grains*, New York, 1935.

TECHNIQUE OF MASS MULTIPLICATION OF *TENOBRACON DEESAE* (CAM.) HYMENOPTERA : BRACONIDÆ FOR USE AGAINST SUGARCANE AND MAIZE BORERS

By E. S. Narayanan and T. V. Venkatraman

Division of Entomology, Indian Agricultural Research Institute, New Delhi)

Received August 23, 1951

(Communicated by Dr. S. V. Desai, F.A.SC.)

Introduction

Stenobracon deesæ (Cam.) is an important larval parasite of the sugarcane top borer *Scirpophaga nivella* F., the stem borers, *Argyria sticticraspis* Hmpsn., *A. tumidicostalis* Hmpsn., *Diatræa auricilia* Dudgn., *D. venosata* Walk., *Sesamia inferens* Walk., *S. uniformis* Dudgn., and the root borer, *Emmalocera depressella* Swinh., as well as the maize and jowar stem borer, *Chilo zonellus* Swinh. It is a large braconid which makes its appearance in sugarcane and maize fields for a short period (June-August) and parasitises a large percentage of larvæ. With an ovipositor longer than its body and measuring more than 16 mm. long, the female parasite (Text-Fig. 1) is endowed with the capacity of reaching borers living in protected situations, especially those inside the rootstock like the root borer (Narayanan, 1938). Though the parasite has apparently a number of hosts, most of them happen to be borers of allied crops so that it can be considered as a potentially effective agent of control of the sugarcane and maize borer complex as a whole.

Attemps to control the sugarcane stem and root borers by large-scale releases of the egg parasite, *Trichogramma evanescens minutum* Riley have given encouraging results in some parts of India. Laboratory studies and field observations have indicated that *S. deesæ* could similarly be successfully used to control the sugarcane and maize borers if a suitable laboratory host on which it can be propagated in quantities were obtainable. As a result of preliminary trials, a suitable host has been discovered in the larva of the "Rice Moth", *Corcyra cephalonica* Stn. The availability of an alternate host for the breeding of *S. deesæ* has opened up encouraging possibilities for releasing it in large numbers in the field, so as to bring about an effective control of the cane and maize borers by the combined use of both egg

and larval parasites. In this paper, the method of breeding the *Stenobracon* parasite in the laboratory on an alternate host is described in some detail.

It may, however, be borne in mind that in the case of *Stenobracon* mass multiplication would merely imply a large-scale breeding and not its production in millions, as is possible in the case of the egg parasite, *Trichogramma*, which by the improved methods recently developed, is now produced, a million a day at a cost of 6·5 dollars per million in the U.S.A. (Spencer, Brown and Phillips, 1935). In fact, the cost of production is only about Rs. 20 per million at the Parasite Laboratory of the Indian Agricultural Research Institute, New Delhi, which compares favourably with the cost of production in U.S.A. Even if the *Stenobracon* parasite can be bred in large numbers and if by properly timed liberations, the natural lack of synchronisation between the populations of pest and parasite can be remedied, the object can be achieved. The releases, however, will have to be combined with that of the egg parasite, *Trichogramma*.

Technique of Mass Culture

Host Material

The first problem was to find out an insect in which *Stenobracon* would oviposit readily in the laboratory and develop, and which could be easily propagated in the laboratory at fairly low cost. Among the factitious hosts tested were the wax moth, *Galleria mellonella* Linn.; the cotton bollworms, *Platedra gossypiella* Saund., and *Earias fabia* Stol.; the mulberry silkworm, *Bombyx mori* Linn.; the potato tuber worm, *Gnorimoschema operculella* (Zell.) and the Rice moth, *Corcyra cephalonica* Stn. The larva of the Rice moth, *C. cephalonica*, only proved to be attractive to the parasite and suitable for its development.

Laboratory-reared host material.—C. cephalonica is mass produced in the Parasite Laboratory of the Indian Agricultural Research Institute, in specially designed cabinets and cages at a very low cost. The eggs of the moth are being used for the mass production of the egg parasite, *Trichogramma*. The full grown larvæ are used to culture *Stenobracon* parasites. Crushed jowar is the host-supporting medium and small quantities of yeast and wheat bran are mixed with jowar to make the medium nutritionally as complete as possible.

Field-collected host material.—In the biological control of some insect pests it is always economical to supplement the mass breeding of hosts by field-collected host material. Field collection of the larvæ of the maize borer, *Chilo zonellus*, has been found to be practical and economical. At the time of harvest of maize crop, the larvæ are found in large numbers in the stems,

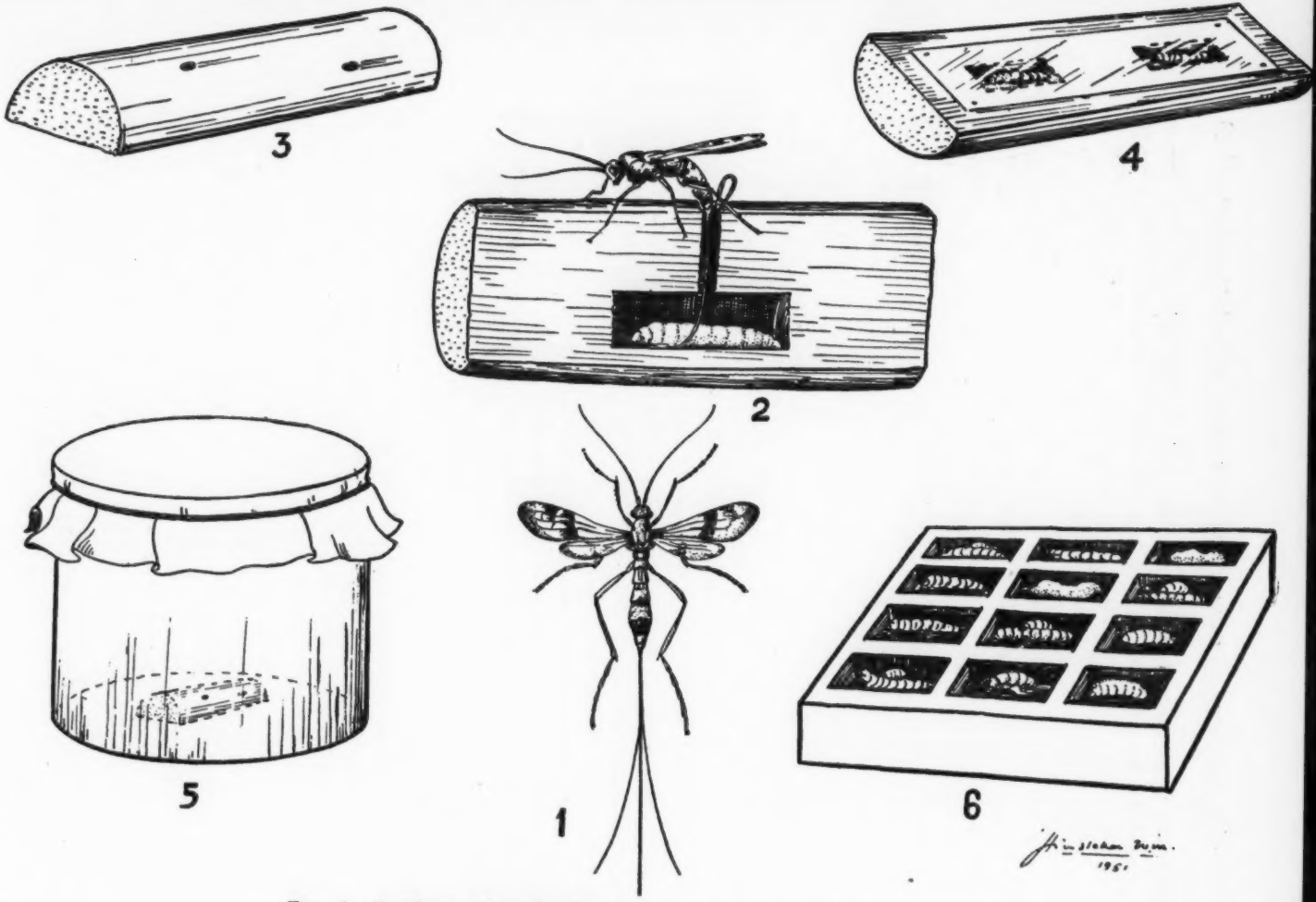


FIG. 1. *Stenobracon deesæ*, female, × 2.
FIG. 2. *Stenobracon* ovipositing on *Corcyra* larva in sugarcane stem.
FIG. 3. Sugarcane stem enclosing factitious host, from above.
FIG. 4. „ „ „ „ seen through celluloid covering below.
FIG. 5. Oviposition jar.
FIG. 6. Wooden block used for rearing parasite grubs.

hibernating in the advanced larval stage. Large quantities of the host material could be collected and stored *in situ* inside maize stems and used for the culture of the parasites as and when required. The stems containing the caterpillars are kept exposed to night temperatures in winter months, by hanging them from the roof of the Parasite Laboratory, which prevents pupation of the caterpillars to some extent. The caterpillars collected and stored in this way can be used for 2–3 months beginning from the month of Septemoer. The hibernating caterpillars of the sugarcane top borer, *S. nivella,* can also be collected in large numbers from the field at the time of sugarcane harvest and stored in a frigidaire at a temperature of about 15° C.

Mass Multiplication of Parasites

Method of offering host larvæ for parasitisation.—Several methods were tried to present the factitious host for parasitisation and from the point of view of maximum parasitisation the method described below has been found most suitable. Pieces of sugarcane stem (Text-Fig. 3) about 3–4″ long and split longitudinally into equal halves, serve very well to enclose the host larvæ. Two to three tunnels (each $\frac{1}{2}'' \times \frac{1}{4}'' \times \frac{1}{4}''$) are prepared in each piece by scooping out the stem from the cut side. The host larvæ are placed in these artificially prepared tunnels, one in each. The caterpillars are prevented from crawling out by means of rectangular pieces of celluloid fixed in position by small pins (Text-Fig. 4). One or two vertical holes about 1·5 mm. in diameter are drilled from the rind towards the tunnel. The parasitised larvæ can be easily made out through the transparent celluloid covering.

Preliminary experiments on the behaviour of the adult females have shown that the odour emanating from the frass of the natural host is a dominant factor in the initial attraction of the female parasite to the host. Hence the external holes on the stem are plugged with frass before exposing them in the oviposition cage. Fresh frass can be easily collected from the field in large quantities and stored in the refrigerator.

Mating.—The parasites, on emergence, are isolated and kept in pairs inside glass tubes (6″ × 2″) open at both ends, for mating. The species mate readily and are fed on 5% sugar solution or preferably on a solution of 1 part of honey and 10 parts of water. The mating tubes are kept in a well lighted place.

Oviposition.—Cylindrical glass jars (4″ × 4″) with muslin covering are very handy and suitable for presenting the host to the parasite (Text-Fig. 5). About 20–24 hours after emergence, the pairs are removed to the

oviposition cages, one pair in each, and sugarcane pieces containing the factitious host are introduced into it. Honey solution is provided in cotton swabs as food for adults. The females are ready for oviposition in about 20 hours after emergence. As soon as the female comes into contact with the piece of stem, it first tries to locate the host inside, by pulsating the antennæ and then begins to lay eggs after stabbing and paralysing the host (Text-Fig. 2). Plate I shows a *Stenobracon* female, caught by the camera, in the act of laying eggs, in the laboratory. Usually only one egg is laid on host but under laboratory conditions, more than one is laid. The oviposition cages are examined twice a day, and the parasitised larvæ are removed and stored in wooden blocks for further development. The rearing jars and muslin covering should be kept clean and free from fungus growth and mites.

Storing of parasitised material.—The parasitised host larvæ containing the eggs and grubs are carefully removed by means of a fine camel-hair brush to a wooden cavity block having rectangular shallow tunnels (Text-Fig. 6). Moistened bits of blotting paper are kept in the wooden cavities on which the parasitised material is placed. The blotting-paper prevents the host larvæ from drying up. If more than one egg is laid on a host, the superfluous eggs are transferred to other paralysed caterpillars on which no egg has been laid. The grubs when full fed, pupate on the blotting-paper and these can be easily removed from the wooden block and stored in petri-dishes for the emergence of parasites.

Development of the grubs.—It is found that more than one full-grown *Corcyra* larva was necessary for the completion of the development of a parasite grub. Several methods were tried to paralyse the host larvæ required for feeding the parasite grub. The codling and amputation methods used by Venkatraman, Negi and Gupta (1948) in the propagation of *Bracon greeni*, were not suitable as the larvæ thus treated decayed and were not liked by the grubs. *Corcyra* larvæ which are already stabbed and paralysed by a different braconid (*Bracon gelechiæ*) have been found quite suitable for this purpose. *B. gelechiæ* readily paralyses host larvæ within a few minutes of exposure and there was no difficulty in obtaining paralysed *Corcyra* larvæ for feeding the grubs. When paralysed *Corcyra* larvæ are offered to the parasite grubs they readily attach themselves to the host and start feeding in a few minutes. Usually two full-grown larvæ are sufficient for the normal development of the parasite grub, but rarely three may be consumed by a grub. Adults bred in this way are quite normal both in size and vigour.

Factors Bearing on the Reproduction of the Parasite in the Laboratory

Regulation of sex ratio.—In the culture of *Stenobracon* on the factitious host (*Corcyra*) there is a very high preponderance of males and a decline in the proportion of females in the successive generations was evident. This appears to be the only drawback in the culture of *Stenobracon* on the factitious host. The factors responsible for affecting the sex ratio is under analysis and one of the apparent causes seems to be the poor quality of the host contents which is again dependent on the nutritional qualities of the host-supporting medium, *i.e.*, jowar. Recently, Flanders (1949) has rightly stressed the need for special attention to nutrition in the case of some entomophagous insects propagated under artificial conditions. A recent advance in this direction is the use of artificial media for the propagation of the host (Theron, 1947). In the case of *Stenobracon*, the normal sex ratio is obtained when the *Corcyra*-bred adults are put back on the natural hosts (*Chilo* or *Argyria*) for breeding. Even under natural conditions the percentage of females is found to be as low as 30. This may be one of the reasons for the low population of the parasite in the field in spite of its polyphagous habit.

Temperature and humidity.—In order to obtain the maximum production per unit of time the rearing temperature and humidity should be such that normal development proceeds with maximum rapidity. A temperature of about 70° F. with about 80% Relative Humidity, is found quite suitable for the production of *Stenobracon*.

Superparasitism and cannibalism.—Under laboratory conditions, when there is a scarcity of host material, more than one egg is laid on a single host and under such conditions cannibalism is prevalent. While examining the parasitised material care is always taken to remove the excess number of eggs and grubs to prevent mortality due to cannibalism.

Our experience during the last three years have shown that if the above precautions are taken, the parasites can be bred in large numbers without any difficulty and the culture maintained in a healthy condition both during the summer and winter months.

Summary

Stenobracon deesæ (Cam.) is a potentially effective larval parasite of sugarcane and maize borers.

The equipment and methods used in the mass propagation of the parasite, *S. deesæ*, are described in some detail. A suitable laboratory host for the propagation of the parasite in the laboratory has been discovered in the

larva of the Rice moth, *Corcyra cephalonica* Stn. The method of exposing the factitious host (*Corcyra*) in artificial tunnels prepared in sugarcane stem is described. Fersh frass of the natural hosts (*Chilo* or *Argyria*) is used to stimulate the female parasite to oviposit on the factitious host, in the laboratory. Laboratory-bred alternate host material is supplemented by field collected natural hosts.

It was found that a single parasite grub requires more than one *Corcyra* larva to complete its development. *Corcyra* larva previously paralysed by another braconid parasite (*Bracon gelechiæ*), is given to the parasite grub, one by one until it becomes full fed.

The percentage of females bred on *Corcyra* is very low and one of the main factors responsible for this, appears to be the nutritionally incomplete quality of the host-supporting medium. The normal percentage of females is restored by putting the *Corcyra*-bred adults to oviposit and breed on the natural hosts.

ACKNOWLEDGMENT

We gratefully acknowledge the interest evinced in this piece of research by Dr. B. P. Pal, Director, Indian Agricultural Research Institute, New Delhi.

REFERENCES

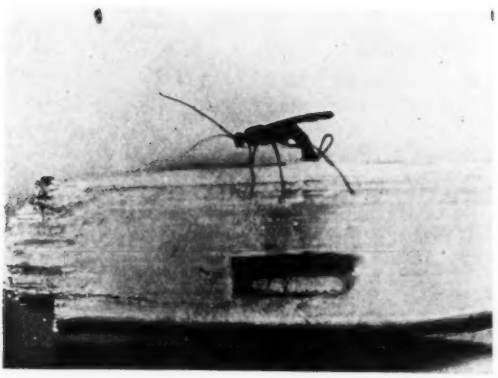
1. Flanders, S. E. .. "Culture of entomophagous insects," *Canad. Ent.*, 1949, **81**, No. II, 257–74.

2. Narayanan, E. S. .. "A Note on *Stenobracon deesæ* (Cam.), a parasite of the root borer of sugarcane," *Ind. J. Agric. Sci.*, 1938, **8**, Pt. II, 215.

3. Spencer, H., Brown, L. and Phillips, M. "New equipment for obtaining host material for the mass production of *Trichogramma minutum*, an egg parasite of various insect pests," *U.S. Dept. Agr. Circ.*, 1935, **376**, 17 pp.

4. Theron, P. P. A. .. "Studies on the provision of hosts for the mass-rearing of codling moth parasites. *Fruit Research* (Tech. Ser. 4)," *Union of S. Afr. Dept. Agr. Sc.* B, 1947, **262**, 45.

5. Venkatraman, T. V., Negi, P. S. and Gupta, S. N. "Preliminary trials in the propagation of *Bracon* (=*Microbracon*) *greeni*, on unnatural hosts," *Proc. Ind. Acad. Sci.*, 1948, **27**, 92–104.



Photograph showing *Stenobracon* in the act of oviposition on the factitious host in sugarcane stem.

SOIL CONDITIONS AND ROOT DISEASES: IV—THE EFFECT OF MICRO-ELEMENTS ON THE OCCURRENCE OF BACTERIA, ACTINOMYCETES AND FUNGI IN SOILS*

By (Miss) C. B. Sulochana

(*University Botany Laboratory, Madras*)

Received December 3, 1951

(Communicated by Prof. T. S. Sadasivan, F.A.Sc.)

Introduction

Work reported earlier by the author (Sulochana, 1952) showed that the activity of Fusaria was markedly diminished by micro-element amendments to soil, whereas under ordinary field conditions, prevention of colonisation of organic matter by Fusaria was found impracticable. This investigation aims at the quantitative estimation of bacteria, actinomycetes and fungi in micro-element amended soils and their possible relationship with the saprophytic activity of Fusaria.

Materials and Methods

The microflora of the soil was assessed by the dilution plate technique (Brierley, *et al.*, 1927). Al, B, Co, Li, Mn, Mo, Ni and Zn at 50, 100, 200 and 400 p.p.m. concentrations were used to amend the soil. 10 g. soil samples from the various treatment series after 1, 2, 3, 4 and 10 weeks, incubation in the laboratory green-house were used for the estimation of bacterial numbers. For counts of actinomycetes and fungi, dilution plates were prepared after 4 weeks' incubation only. Thornton's agar (Thornton, 1922) was employed for bacteria and actinomycetes, and potato-dextrose agar with 0·2% H_3BO_3 (Tyner, 1944) for fungal counts. A series of four plates in parallel was maintained for each treatment and 1 ml. of 10^{-5}, 10^{-4} and 10^{-3} dilutions of soil samples were used for bacteria, actinomycetes and fungi respectively.

The experimental design had to be made complex so as to test simultaneously the effect of several distinct treatment series and their reaction on one another. Detailed statistical analyses were carried out as an essential preliminary to an accurate appreciation of the factors responsible for any apparent difference between treatments or combination of treatments under

* Part of thesis approved for the Degree of Master of Science of the Madras University.

TABLE

Bacterial numbers in millions in 10 gm. of air-dry soil—

Micro-element	Incubation period in weeks	CONCENTRATION							
		Control 0				50 P.P.M.			
		1	2	3	4	1	2	3	4
Aluminium ..	1	69	73	77	62	42	69	56	64
	2	56	94	102	74	41	32	36	48
	3	65	58	79	84	60	62	76	57
	4	78	80	88	70	64	64	56	65
	10	92	84	73	86	74	92	80	84
Boron ..	1	69	73	77	62	65	67	58	71
	2	56	94	102	74	94	96	88	86
	3	65	58	79	84	116	118	90	99
	4	78	80	88	70	117	120	198	106
	10	92	84	73	86	94	138	142	128
Cobalt ..	1	69	73	77	62	40	42	34	32
	2	56	94	102	74	42	54	71	43
	3	65	58	79	84	65	78	70	49
	4	78	80	88	70	44	49	50	46
	10	92	84	73	86	70	74	65	61
Lithium ..	1	69	73	77	62	93	92	90	84
	2	56	94	102	74	82	78	64	81
	3	65	58	79	84	80	80	78	84
	4	78	80	88	70	96	115	108	98
	10	92	84	73	86	120	136	148	131
Manganese ..	1	69	73	77	62	96	140	108	121
	2	56	94	102	74	101	126	109	107
	3	65	58	79	84	140	130	136	108
	4	78	80	88	70	156	188	170	180
	10	92	84	73	70	240	118	176	143
Molybdenum ..	1	69	73	77	62	116	121	98	109
	2	56	94	102	74	81	84	80	71
	3	65	58	79	84	99	92	86	103
	4	78	80	88	70	75	90	82	78
	10	92	84	73	70	150	142	108	131
Nickel ..	1	69	73	77	62	30	28	10	16
	2	56	94	102	74	31	36	21	24
	3	65	58	79	84	40	44	42	36
	4	78	80	88	70	56	43	45	54
	10	92	84	73	70	51	40	26	34
Zinc ..	1	69	73	77	62	74	66	39	71
	2	56	94	102	74	49	67	68	68
	3	65	58	79	84	50	76	73	56
	4	78	80	88	70	116	121	86	106
	10	92	84	73	70	108	96	98	101

I

Udamalpet "Wilt-sick" soil amended with micro-elements

OF MICRO ELEMENT

100 P.P.M.				200 P.P.M.				400 P.P.M.			
1	2	3	4	1	2	3	4	1	2	3	4
58	40	42	31	10	15	14	9	9	8	12	9
36	37	52	49	12	6	7	11	8	6	15	14
45	48	25	42	28	31	22	25	39	35	34	35
41	42	36	38	31	33	32	27	18	16	15	14
60	65	58	56	40	42	38	41	22	16	31	15
91	84	75	65	60	42	43	54	30	38	46	51
101	108	92	103	70	76	56	54	36	42	50	46
127	79	115	122	65	58	80	89	60	66	74	81
120	125	142	118	96	100	110	94	80	88	84	86
141	156	99	136	91	95	99	84	60	65	45	58
35	26	38	42	21	18	17	26	20	21	24	12
50	54	42	41	39	40	48	20	21	24	14	30
59	45	65	52	36	24	49	51	40	25	27	27
40	33	34	41	30	32	35	27	26	25	24	26
50	40	48	42	34	33	34	26	18	24	46	22
63	84	76	88	52	45	56	41	42	41	36	42
75	56	76	71	37	29	24	41	40	15	36	35
60	54	61	75	41	45	54	60	41	33	37	33
80	100	86	92	66	65	74	80	35	57	48	38
106	96	88	94	70	120	64	65	41	42	46	52
130	135	99	118	96	90	93	108	74	88	98	96
115	120	123	108	99	193	108	84	80	84	105	96
190	176	165	180	90	99	105	115	80	46	97	111
237	220	208	195	126	130	135	140	105	96	110	99
108	126	131	134	90	94	108	116	96	99	84	88
80	84	96	91	53	54	48	42	30	42	33	31
70	64	51	49	48	46	51	59	28	26	40	36
80	84	76	94	60	50	48	51	36	33	28	43
71	84	88	101	51	60	68	42	26	48	50	31
96	98	114	92	68	75	69	101	30	38	54	47
29	26	31	42	6	8	12	11	5	3	10	14
21	24	20	12	10	6	14	7	11	4	8	9
38	40	41	34	15	10	12	16	10	9	9	14
38	50	41	43	21	24	26	22	20	19	22	18
33	48	42	45	20	18	6	16	17	22	10	11
67	69	49	56	40	42	44	38	31	32	36	30
66	80	74	49	45	44	39	48	36	46	31	46
80	81	65	67	40	44	43	39	30	62	36	43
76	80	84	88	63	64	70	56	60	65	41	59
75	82	56	88	42	49	50	66	42	20	21	22

observation. As one series of treatment was superimposed on a second, it was necessary to take into consideration not only the straightforward treatment comparisons, but also the way in which the combination of treatments reacted on one another. The whole unit sums of squares included the main factors, namely, micro-elements, concentrations and incubation periods and their respective error variance factors, by means of which subtle differences amongst the components of each factor were tested (Paterson, 1939).

EXPERIMENTAL RESULTS

Bacteria

Tabulated statements of the results obtained in this study are included in Tables I, II and III (*a*, *b*, *c*).

TABLE II

Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Log_{10} V	Value of Z		
					Calculated	at 5% point	at 1% point
Micro-element ..	7	431636·17	61662·31	4·7900197	2·37378	·3885 (6,120)*	·5419 (6,120)
Concentrations ..	4	268943·39	67235·847	4·8276006	2·41705	·4475 (4,120)	·6234 (4,120)
Incubation period	4	49836·84	12459·21	4·0954902	1·57417	·4475 (4,120)	·6234 (4,120)
INTERACTIONS:							
Elements × concentrations	28	121441·04	4337·18	3·6372074	1·04655	·2376 (24,120)	·3339 (24,120)
Concentrations × incubation periods	16	1671·45	104·59	2·0194902	..	..	..
Elements × incubation periods	28	101997·77	3642·77	3·5614318	0·95931	·2376 (24,120)	·3339 (24,120)
Elements × concentrations × incubation periods	112	24177·76	214·98	2·3323981	..	..	..
Error ..	600	320904·96	534·84	2·7281947	..	..	..
Total ..	799	1320609·38					

* Degrees of freedom for treatments and error (nearest values from ' Z ' table.)

The response of soil bacteria to micro-element treatments was found to be strikingly significant.

It could be inferred from the Analysis of Variance that micro-elements, concentrations and the periods of incubation were highly significant, and of the three sets of second order interactions, two were highly significant. They were (*a*) Interaction between micro-elements and concentrations, (*b*) Interaction between micro-elements and incubation periods.

The interaction between concentrations and incubation periods and the triple interaction were found to be insignificant.

Based on the diverse effects of the micro-element amendments, the micro-elements were grouped together in the following order: Ni, Al, Co, Zn, Li, Mo, B and Zn (Sadasivan, 1951, Text-Figs. 5 and 6, pp. 144–45).

The results indicated adverse effects of Ni, Al and Co at all levels on the population of bacteria in the amended soil over the control series. In the interaction: micro-element × incubation periods, bacterial numbers were highest at 50 p.p.m. in the four concentrations tried.

In Zn, Li and Mo, the increase or decrease of bacterial numbers over that of the control depended to a large extent on the concentration as well as the incubation period. Thus, when incubation periods were fixed, the numbers recorded after 10 weeks were considerably higher than control figures at 50 and 100 p.p.m.

In the 2nd series of Mo treatments at 50 p.p.m., bacterial numbers were highest followed closely by 100 p.p.m. This was noticeable in advanced incubation periods at 100 p.p.m. of Li, whereas at 50 p.p.m. Zn, a similar effect was seen after 4 and 10 weeks' incubation.

The effects of B and Mn on bacterial population were considerable at all concentrations. Records made after 4 weeks' incubation showed very great increase in bacterial numbers and the maximum was found at 100 p.p.m. A mean value of 215 millions of bacterial colonies was recorded at this point, whereas the control figure was only 79 millions. Boron at 50 and 100 p.p.m. concentrations considerably increased the bacterial numbers. The highest increase recorded in this case was 133 millions at 100 p.p.m. at the end of 10 weeks' incubation.

The results of the whole-unit treatment analyses are presented in Tables III *a*, *b* and *c*.

TABLE III *a*

Analysis of variance

Factor	Degree of freedom	Sum of squares	Variance
Whole-unit treatment ..	31	434428·18	
Micro-elements ..	7	431636·17	61662·31
Error ..	24	2792·01	116·33

Critical differences at 5% level

$= \sqrt{116{\cdot}33 \times 2 \times 100} \times 2{\cdot}064 = 314{\cdot}9.$

TOTAL COUNTS

Manganese (A) ..	11324	Zinc (E) ..	6327
Boron (B) ..	8536	Cobalt (F) ..	4625
Molybdenum (C) ..	7077	Aluminium (G) ..	4619
Lithium (D) ..	6982	Nickel (H) ..	3474

A B C D E F G H

TABLE III *b*

Analysis of variance

Factor	Degree of freedom	Sum of squares	Variance
Whole-unit treatment ..	19	295669·41	
Concentrations ..	4	268943·39	67235·85
Error ..	15	26726·02	1781·73

Critical difference at 5% level

$= \sqrt{1781 \cdot 73 \times 2 \times 160} \times 2 \cdot 131 = 1609.$

TOTAL COUNTS

Control (B) ..	12352	200 p.p.m. (D) ..	8285
50 p.p.m. (A) ..	13624	400 ,, (E) ..	6456
100 ,, (C) ..	12247		

A B C D E

TABLE III *c*

Analysis of variance

Factor	Degree of freedom	Sum of squares	Variance
Whole-unit treatment ..	19	181688·38	
Incubation period ..	4	49836·84	12459·21
Error ..	15	131851·54	8790·10

Critical difference at 5% level

$= \sqrt{8790 \cdot 1 \times 2 \times 160} \times 2 \cdot 131 = 3575.$

TOTAL COUNTS

One week (E) ..	9042	Four weeks (B) ..	12006
Two weeks (D) ..	9437	Ten weeks (A) ..	12076
Three weeks (C) ..	10403		

A B C D E

(*a*) The analysis showed a significant difference between most of the elements and the order with reference to the total counts were:

$$Mn > B > Mo \gg Li > Zn > Co \gg Al > Ni.$$

(*b*) In the comparable total counts of the bacterial colonies in relation to concentrations, the order of efficiency was:

50 p.p.m. > control > 100 p.p.m. > 200 p.p.m. > 400 p.p.m.

(*c*) The comparable total counts for the five incubation periods tried did not show any significant difference between these factors. However, the total counts of bacterial colonies were in the order:

10 weeks > 4 weeks > 3 weeks > 2 weeks > 1 week.

Actinomycetes

Table IV gives the data for actinomycetes developed on agar plates rom micro-element amended soil samples.

TABLE IV

Actinomycetes in ten thousands per gram of air-dry soil—'wilt-sick' soil amended with micro-elements

Micro-elements	Concentration of Micro-elements																			
	Control				50 p.p.m.				100 p.p.m.				200 p.p.m.				400 p.p.m.			
	Replicates				Replicates				Replicates				Replicates				Replicates			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Aluminium ..	4	2	4	5	4	4	6	3	5	2	4	5	8	3	8	4	3	8	4	2
Boron ..	4	2	4	5	3	2	4	7	4	4	2	6	4	3	8	6	5	4	4	7
Cobalt ..	4	2	4	5	8	8	3	6	7	6	3	6	3	2	13	8	3	3	3	4
Lithium ..	4	2	4	5	3	2	1	5	10	20	12	8	8	10	14	21	8	7	8	11
Manganese ..	4	2	4	5	4	5	3	6	3	3	4	3	3	2	1	3	3	4	3	3
Molybdenum ..	4	2	4	5	2	1	3	4	1	1	2	4	3	3	2	1	3	2	4	1
Nickel ..	4	2	4	5	1	2	1	1	1	1	1	1	2	1	1	2	2	1	1	1
Zinc ..	4	2	4	5	2	3	1	1	2	2	2	1	3	1	1	1	1	2	2	1

TABLE V

Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Log_{10} V	Value of Z		
					Calculated	at 5% point	at 1% point
Micro-elements ..	7	583·55	83·36	1·9209577	1·53886	·3885 (6,120)*	·5152 (6,120)
Concentrations ..	4	36·73	9·18	0·9628427	·435780	·4475 (4,120)	·6234 (4,120)
INTERACTION :							
Elements × concentrations	28	460·50	16·4	1·2148438	·72591	·2376 (24,120)	·3339 (24,120)
Error ..	120	461·71	3·84	0·5843312			
Total ..	159	1542·49					

* Degrees of freedom for treatments and error (nearest values from 'Z' table).

TABLE V *a*

Analysis of variance

Factor	Degrees of freedom	Sum of squares	Variance
Whole-unit treatment ..	31	669·15	
Micro-elements ..	7	583·55	83·36
Error ..	24	85·60	3·56

Critical difference at 5% level

$$= \sqrt{3 \cdot 56 \times 2 \times 20} \times 2 \cdot 175 = 26 \cdot 15.$$

TOTAL COUNTS

Lithium (A) ..	163	Manganese (E) ..	68
Cobalt (B) ..	101	Molybdenum (F) ..	52
Aluminium (C) ..	88	Zinc (G) ..	41
Boron (D) ..	88	Nickel (H) ..	35

A B C D E F G H

The inferences that could be drawn from the Analyses of Variance are:

(*a*) The micro-elements influenced significant effect on the occurrence of actinomycetes. Significant factors were (i) micro-elements and (ii) the interaction between the micro-elements and concentrations.

TABLE V *b*

Analysis of variance

Factor	Degree of freedom	Sum of squares	Variance
Whole-unit treatment ..	19	124·95	
Concentrations ..	4	36·73	9·18
Error	15	88·22	5·88

Critical difference at 5% level

$= \sqrt{5{\cdot}88 \times 2 \times 32} \times 2{\cdot}048 = 39{\cdot}79.$

TOTAL COUNTS

Control (C) ..	120	200 p.p.m. (A) ..	153
50 p.p.m. (E) ..	109	400 ,, (D) ..	118
100 ,, (B) ..	136		

A B C D E

(*b*) Lithium at 100 and 200 p.p.m. stimulated the development and occurrence of actinomycetes to a considerable extent. Co, Al, Mn and B showed slight increase in the numbers of actinomycete colonies over that of the control. In all other cases the numbers of actinomycetes were found to be less than the control.

The data recorded (Table VI) on statistical analyses (Table VI *a*) showed the effects of micro-elements, concentrations and the interaction between these factors to be highly significant both at 1 and 5 % points.

1. The micro-element amendments to soil enormously increased the fungal numbers in the soil with the exception of Zn and Ni.

2. Grouping the elements on their relative influence in increasing the fungal numbers would be as follows:

Li, Mn, Mo, Co, B and Al in the descending order. Significant differences were observed between Li and other micro-elements closely following as indicated above.

3. Fungal numbers showed fivefold increase in Li treated soil and as much as three times and more over the control, in the case of Mn, Mo, Co, B and Al.

Fungi

TABLE VI

Fungi in thousands per gram of air-dry soil—'wilt-sick' soil amended with micro-elements

Micro-elements	CONCENTRATION OF MICRO-ELEMENTS																			
	Control (=zero concentration)				50 p.p.m.				100 p.p.m.				200 p.p.m.				400 p.p.m.			
	Replicates				Replicates				Replicates				Replicates				Replicates			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Aluminium ..	6	4	5	5	4	5	5	3	5	6	7	9	22	21	23	16	12	18	14	15
Boron ..	6	4	5	5	25	12	16	15	12	16	18	11	6	16	24	8	10	11	12	10
Cobalt ..	6	4	5	5	13	8	6	6	15	8	8	10	14	20	13	12	27	26	14	29
Lithium ..	6	4	5	5	13	11	10	9	28	45	20	15	23	16	17	22	14	12	10	10
Manganese ..	6	4	5	5	28	10	12	16	14	5	3	17	21	24	15	22	14	6	10	16
Molybdenum ..	6	4	5	5	20	14	17	11	6	14	15	9	10	20	21	16	16	18	9	12
Nickel ..	6	4	5	5	2	3	4	3	3	2	2	1	5	3	2	2	1	3	2	3
Zinc ..	6	4	5	5	2	8	8	5	8	6	6	7	5	4	2	6	4	3	3	2

TABLE VI *a*

Source of variation	Degree of freedom	Sum of squares	Mean squares	Log_{10} V	Value of Z		
					Calculated	at 5% point	at 1% point
Micro-elements ..	7	2557·45	365·35	2·5627091	1·56383	·3696	·5158
Concentrations ..	4	1405·54	351·39	2·5457894	1·54435	·4930	·6234
INTERACTION :							
Elements × concentrations	28	2443·46	87·27	1·940865	·84790	·1131	·1612
Error ..	120	1920·75	16·01	1·2043913			
Total ..	159	8327·20					

TABLE VI *b*

Analysis of variance

Factor	Degrees of freedom	Sum of squares	Variance
Whole-unit treatment ..	31	2771·60	
Micro-elements ..	7	2557·45	365·35
Error ..	24	214·15	8·92

Critical difference at 5% level

$= \sqrt{8{\cdot}92 \times 2 \times 20} \times 2{\cdot}179 = 32{\cdot}7.$

TOTAL COUNTS

Lithium (A) ..	295	Boron (E) ..	242
Manganese (B) ..	253	Aluminium (F) ..	205
Molybdenum (C) ..	248	Zinc (G) ..	99
Cobalt (D) ..	244	Nickel (H) ..	61

A B C D E F G H

TABLE VI *c*

Analysis of variance

Factor	Degrees of freedom	Sum of squares	Variance
Whole-unit treatment ..	19	1643·45	
Concentrations ..	4	1405·54	351·39
Error ..	15	237·91	15·86

Critical difference at 5% level

$= \sqrt{15{\cdot}86 \times 2 \times 32} \times 2{\cdot}048 = 66{\cdot}09.$

TOTAL COUNTS

Control (E) ..	160	200 p.p.m. (A) ..	451
50 p.p.m. (D) ..	324	400 ,, (B) ..	361
100 ,, (C) ..	351		

A B C D E

Discussion

Many cases are known of a population of one species of micro-organism or a group of micro-organisms in soil affecting the numbers or the metabolic activity of a population of some other species or groups of microflora (Alexopoulos and Herrick, 1942; Alexopoulos, *et al.*, 1938; Allen and Haensler, 1935; Daines, 1937; D'aeth, 1939; Van Luijk, 1938 *a*, 1938 *b* and Waksman, 1941).

Comparatively very little work has been done on the effects of additions of trace-elements to soil on the general microbial flora. Hanna and Purvis (1940) found that the total number of micro-organisms in soil was greatly increased by the addition of borax, especially with higher soil reaction, pH 7·7. Iyer and Rajagopalan (1938) have reported that Mn hastens decomposition of organic matter, thereby increasing the availability of plant food. Vlasynk (1937) also reported that Mn expedites the decomposition of organic matter in the soil, stimulates micro-biological processes and increases moisture. Chandler (1937) and Millikan (1938, 1942) reported that Zn influences the activities of the soil microflora. Results of Burk and Herner (1936), Van Niel (1935) and Birsch-Hirschfeld (1932) on the effect of molybdenum and vanadium on nitrogen fixation by *Azotobacter* and *Rhizobia* of legumes indicate that Mo may perform a role in the symbiotic nitrogen fixation by legumes.

The increases in the colonies of bacteria in Mn and B treatments were considerable, while Mo, Li and Zn stimulated increase in bacterial colonies at lower concentrations. Ni, Al and Co lowered the bacterial numbers to striking degrees. These findings suggested an essentiality of certain of the micro-elements for increased metabolic activity of the soil bacteria and a few appeared to bring about a partial negation of the microbial activity.

In the microcosm of the soil, actinomycetes and fungi form the next major groups of micro-organisms. Experimental results indicated that Li increased the numbers of actinomycetes in soil. Micro-element amendments with the exception of Zn and Ni increased the fungal numbers considerably.

These results will be found significant from the view-point of (*a*) increasing the competition for the available sources of energy in the soil substratum under modified soil conditions and (*b*) as a possibility of bringing about intense microbial antagonism—anti-fungal and anti-bacterial. The principles of biological control of soil-borne plant diseases were outlined by Garrett (1944) in terms of the soil population in a state of dynamic equilibrium. In these trials, a correlation could be found between the effects

of micro-element amendments on the saprophytic potentialities (Sulochana, 1952) on the one hand and the general microflora on the other. Those amendments which favoured increased activities of the microbial population accelerated the loss of viability of the pathogen, while other micro-elements exerted direct toxic effects on soil Fusaria. The latter view was more or less confirmed by the results obtained in the study on the germination of conidia of *Fusarium vasinfectum* when introduced into the micro-element amended soil, as evidenced by the marked inhibition on spore germination (Sulochana, 1952).

Summary

A study on the effects of certain micro-elements—Al, B, Co, Li, Mn, Mo, Ni and Zn—on soil bacteria, actinomycetes and fungi in the soil has been made.

The occurrences of these groups of micro-organisms were found to be significantly influenced by the amendments.

The bacterial numbers increased enormously in the Mn amended soils. This was closely followed by B, and by Zn, Mo and Li to a lesser extent. Ni, Al and Co exerted an adverse influence on the bacterial population of the soil.

The statistical interpretation of the results clearly indicated the marked significance of all the three factors, *viz.*, Micro-elements, concentrations, incubation periods and also the following interactions:

Micro-elements × Concentrations

Micro-elements × Incubation periods

The numbers of actinomycete colonies were observed in greater numbers in soil samples amended with Li and to a lesser extent with Mn, B and Co.

The micro-elements with the exception of Zn and Ni increased considerably fungal numbers in the soil. Li showed fivefold increase in fungal numbers and as much as threefold increase in Mn, Mo, Co, B and Al as compared with the unamended control.

Acknowledgements

The author wishes to express her indebtedness to Prof. T. S. Sadasivan. Grateful thanks are due to Mr. D. V. Rajalakshman for assistance in the statistical analysis of the data and to the University of Madras for award of a stipend during the tenure of which this investigation was conducted.

REFERENCES

Alexopoulos, C. J. and Herrick, J. A. "Studies in antibiosis between bacteria and fungi, III. Inhibitory action of some actinomycetes on various species of fungi in culture," *Bull. Torrey bot. Club*, 1942, **69**, 247–51.

———, Arnett, R. and McIntosh, A. V. "Studies in antibiosis between bacteria and fungi," *Ohio J. Sci.*, 1938, **38**, 221–35.

Allen, M. C. and Haenseler, C. M. "Antagonistic action of *Trichoderma lignorum* on *Rhizoctonia* and other soil fungi," *Phytopathology*, 1935, **25**, 244–52.

*Birsch-Hirschfeld, L. .. "The influence of molybdenum and soil extract matter on nitrogen fixation with *Azotobacter chroococcum*," *Arch. microbiol.*, 1932, **8**, 341–61.

Brierley, W. B., Jewson, S. T. and Brierley, M. "The quantitative study of soil fungi," *Proc. International Congress of Soil Science*, 1927, **3**, pp. 24.

Burk, D. and Herner, K. C. "The role of traces of molybdenum in the physiology and agrobiology of *Azotobacter*," *Soil. Sci. Amer. Proc.*, 1936, **1**, 213–14.

Chandler, W. H. .. "Zinc as a nutrient for plants," *Bot. Gaz.*, 1937, **98**, 625–46.

D'aeth, H. R. H. .. "A survey of interaction between fungi," *Biol. Rev.*, 1939. **14**, 105–31.

Daines, R. H. .. "Antagonistic action of *Trichoderma* on *Actinomyces scabies* and *Rhizoctonia solani*," *Amer. Potato J.*, 1937, **14**, 85–93.

Garrett, S. D. .. *Root Disease Fungi*, Chronica Botanica Co., Waltham Mass., U.S.A., 1944.

Hanna, W. J. and Purvis, E. J. .. "Effect of borax and Lime on micro-organisms," *Soil Sci.*, 1940, **52**, 275–79.

Iyer, C. R. H. and Rajagopalan, R. "Role of manganese in soil fertility," *Abs. Proc. 25th Indian Sci. Cong.*, 1938, 227.

Millikan, C. R. .. "A preliminary note on the relation of zinc to diseases in cereals," *J. Dept. Agric. Vict.*, 1938, **36**, 409–16.

———— .. "Studies on soil conditions in relation to root-rot of cereals," *Proc. Roy. Soc. Vict. N. S.*, 1942, **54**, 145–95.

Paterson, D. D. .. *Statistical Technique in Agricultural Research*. McGraw-Hill Book Company, Inc., New York and London, 1939.

Sadasivan, T. S. .. "Role of trace-elements in control of root-infecting fungi," *Proc. Indian Acad. Sci.*, 1951, **33B**, 135–49.

Sulochana, C. B. .. "Soil conditions and root diseases, III, with special reference to survival and colonization of soil Fusaria in micro-element amended soils." *Proc. Indian Acad. Sci.*, 1952, **39 B**, 209–13.

———— .. "Soil conditions and root diseases, V. Germination of conidia of *Fusarium vasinfectum* in micro-element amended soils (unpublished).

Thornton, H. G. .. "On the development of standardized agar medium for counting soil bacteria with special reference to the repression of spreading colonies," *Ann. appl. Biol.*, 1922, **9**, 241–74.

Tyner L. E. .. "Effect of media composition on the numbers of bacterial and fungal colonies developing on Petri dishes," *Soil Sci.*, 1944, **57**, 271–74.

*Van Luijk, A. .. "Antagonism between various micro-organisms and different species of the genus *Pythium* parasitising upon grass and lucerne," *Meded. Phytopath. Lab. Scholten*, 1938 *a*, 14, 45–83.

———— .. "Antagonism of *Penicillium* species on *Pythium de Baryanum*," *Chron. bot.*, 1938 *b*, 4, 216–21.

*Van Niel, C. B. .. "A note on the apparent absence of *Azotobacter* in soils," *Arch. Microbiol.*, 1935, 6, 215–18.

*Vlasynk, P. A. .. "Manganese fertilizer," *Principal results, scientific studies, V.N.I.S. for* 1936, 52–55; (*Chemical Abstracts*, 1937, 34, 2997).

Waksman, S. A. .. "Antagonistic relations of micro-organisms," *Bact. Rev.*, 1941, 5, 231–91.

* Original not seen.

EMBRYOLOGY OF *ANAGALLIS PUMILA* SWARTZ.

By M. V. S. Raju

(*Department of Botany, Central College, Bangalore*)

Received March 23, 1952

(Communicated by Professor L. Narayan Rao)

Introduction

The genus *Anagallis*, a member of the Primulaceæ, is placed by Engler and Prantl (1889) in the tribe Samoleæ, sub-tribe Lysimachiæ—Angallidinæ. Hooker (1879) includes this genus in the tribe Lysimachieæ. Gamble (1925) has reported two species of *Anagallis* from South India. The present account deals with the embryology of one of them, *viz.*, *Anagallis pumila*. Dahlgren (1916) has described certain aspects of the embryology of some members of this family and the closely allied families, Myrsinaceæ and Theophrastaceæ. Souéges (1937) has described in detail the embryogeny of *Samolus Valerandi* L.

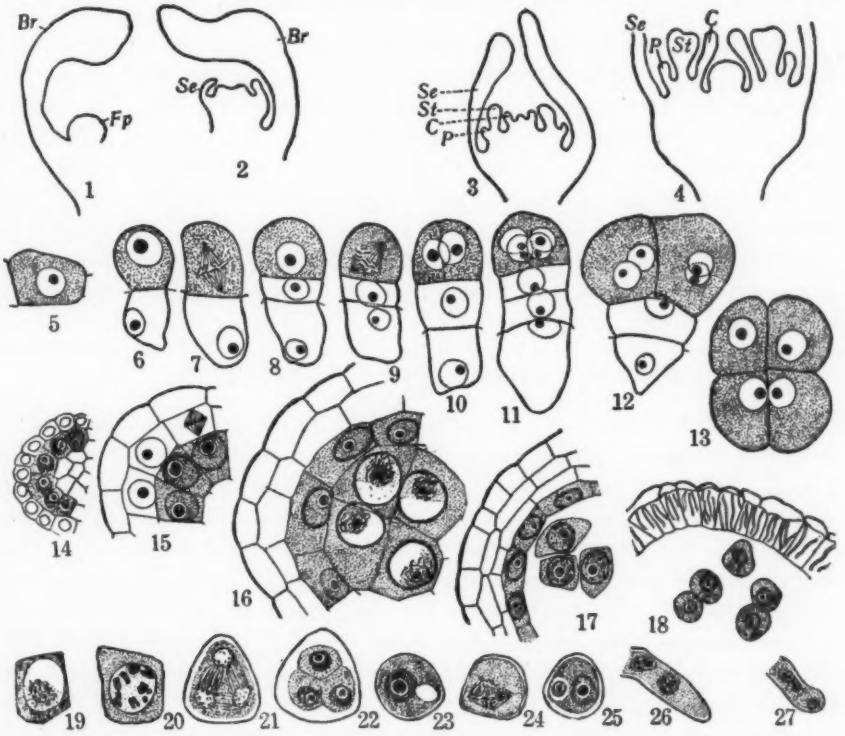
The material was collected in Government Botanical Gardens, Bangalore, and fixed in Formalin-acetic-alcohol. Customary methods were followed and sections were cut at a thickness of 10–16 microns. They were stained in Heidenhain's iron-alum-hæmatoxylin with eosin as counter-stain. Some sections were also stained in safranin and fast green.

Flower

The floral parts take their origin in the following succession: sepals, stamens, carpels and petals (Figs. 1–4). Glandular hairs are present on the wall of the ovary, at the base of the stamens, on the calyx and corolla. An epidermal cell becomes conspicuous by its dense contents and prominent nucleus (Fig. 5). It divides transversely producing an outer cell and an inner cell (Fig. 6). The inner cell gets merged with the remaining cells of the epidermis. The outer cell divides transversely to form two superposed cells (Figs. 7 and 8). Of these the upper cell divides vertically (Figs. 9 and 10) and by one more division at right angles to the first produces a four-celled gland (Figs. 11–13). The lower cell forms the stalk, which is sometimes two-celled (Fig. 11). The mature glandular hair consists of four cells borne on a unicellular stalk (Fig. 12). The cells of the gland are large and enclose prominent nuclei and dense contents.

Microsporangium and Male Gametophyte

A transverse section of a young anther lobe shows a plate of six hypodermal archesporial cells (Fig. 14). These divide periclinally to form the primary parietal layer and primary sporogenous layer (Fig. 15). By further periclinal divisions in the primary parietal layer the wall of the anther is



Figs. 1–27.—Figs. 1–4. L.S. young flower buds showing the development of floral parts, × 65. *Fp* = Floral primordium. *Br* = Bract. *Se* = Sepal. *St* = Stamen. *C* = Carpel. *P* = Petal. Figs. 5–12. Stages in the development of glandular hairs, ×900. Fig. 13. Surface view of glandular hair, × 900. Fig. 14. Portion of the anther lobe showing hypodermal archesporial cells, ×450. Fig. 15. Portion of the anther showing primary parietal and sporogenous layers, × 900. Fig. 16. Portion of the anther showing epidermis, endothecium, uninucleate tapetum and sporogenous cells, ×970. Fig. 17. Later stage, ×900. Fig. 18. Portion of mature anther showing the irregular epidermis, fibrillar endothecium and two-celled pollen grains, ×450. Figs. 19–20. Early stages in the division of microspore mother cells, ×1,800. Fig. 21. Simultaneous division of microspore mother cell, ×900. Fig. 22. Tetrad of microspores, × 900. Fig. 23. Uninucleate microspore, × 900. Fig. 24. First division in the microspore, × 900. Fig. 25. Two-celled pollen grain showing a large tube cell and a small generative cell, × 900. Figs. 26–27. Pollen tubes showing the degeneration of the tube nucleus and the division of the generative nucleus, × 900.

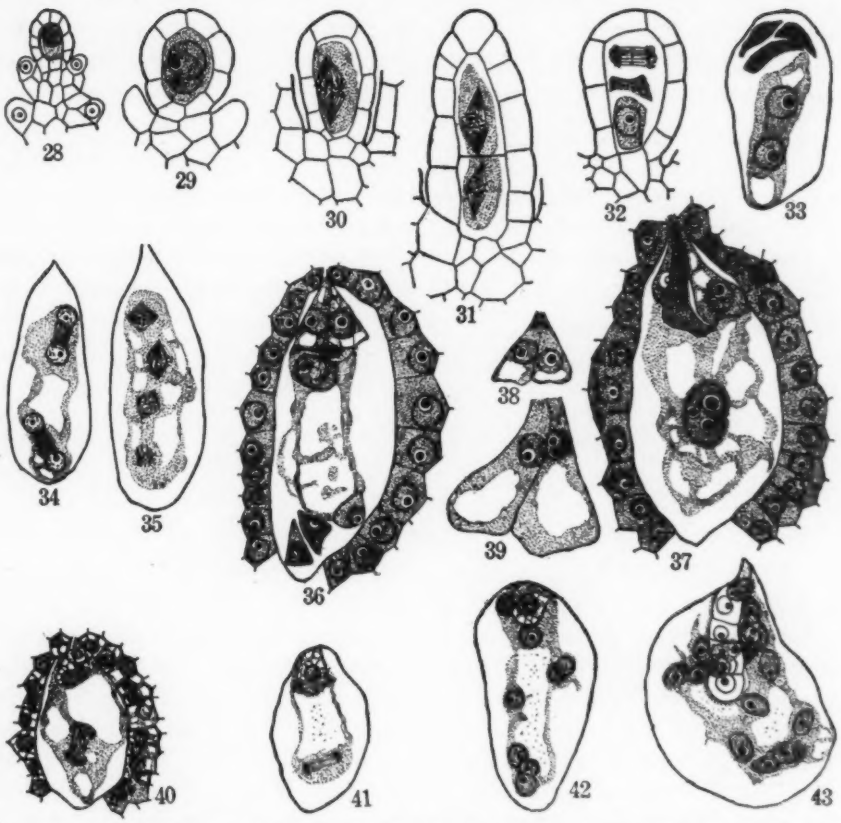
formed (Figs. 15–17). It consists of an epidermis, endothecium, middle layer and the tapetum. The tapetal cells remain uninucleate throughout (Fig. 17) and are of the glandular type. The microspore mother cells round off, undergo the usual reduction divisions and form tetrads of microspores (Figs. 19–22). Quadripartition of the microspores takes place by cleavage furrowing and the microspores are arranged tetrahedrally (Figs. 21–22).

In the mature anther the middle layer and the tapetum completely disorganise. The endothecium develops the usual fibrillar thickenings and the epidermal cells present a shrivelled appearance (Fig. 18). The young pollen grain is uninucleate and its nucleus soon moves towards the wall by the formation of a vacuole (Fig. 23). There it divides (Fig. 24) to form a small generative cell and a large tube cell (Fig. 25) and this is the shedding stage of the pollen grains. The tube nucleus degenerates in the pollen tube and the generative cell divides to form two male cells (Figs. 26 and 27). Cases were also observed where the pollen grains had germinated *in situ* within the anther locule.

MEGASPORANGIUM AND FEMALE GAMETOPHYTE

The ovary is superior, unilocular, with indefinite number of anatropous, bitegmic ovules arranged on a globose free central placenta. From this typical anatropous condition of the ovule, there is a gradual change to apotropy and even campylotropy as in other members of this family (Dahlgren, 1916). The hypodermal archesporium is differentiated in the young nucellar primordium before the origin of the integuments. Sometimes two archesporial cells are also met with in the same nucellus (Fig. 29). Soon the initials of the integuments are laid down (Fig. 28) and they grow round the nucellus. To start with both the integuments are two-layered. Later the inner layer of the inner integument divides periclinally so as to make it three-layered. The outermost layer of the outer integument and the innermost layer of the inner integument are filled with tannin. Dahlgren (1916) also reported two-layers of cells in the outer integument of *Hottonia*, *Samolus* and others. Decrock (quoted in Dahlgren, 1916) observed in *Douglasia vitaliana* a three-layered outer integument, but this is considered doubtful by Dahlgren. In *Cortusa Matthioli* and *Primula officinalis*, Dahlgren describes a many layered inner integument. The micropyle is organised by both the integuments.

The archesporial cell functions directly as the megaspore mother cell (Fig. 30) and it undergoes the usual divisions to form a linear tetrad (Fig. 31). Sometimes a T-shaped tetrad is also met with (Fig. 32). The lowermost



FIGS. 28–43.—Fig. 28. L.S. young nucellus showing archesporium, ×450. Fig. 29. Double archesporial cells, ×900. Fig. 30. Megaspore mother cell in division, ×900. Fig. 31. Dyad cells in division, ×900. Fig. 32. T-shaped tetrad, ×900. Figs. 33–35. First, second and third divisions in the formation of embryo sac, ×900. Fig. 36. Mature embryo sac, × 900. Fig. 37. A stage in double fertilisation, × 900. Fig. 38. Young synergids, ×485. Fig. 39. Older stage of same, × 485. Figs. 40–42. Stages in the formation of the free nuclear endosperm. × 450. Fig. 43. Embryo sac with four-celled preembryo and sixteen free endosperm nuclei, ×4,500.

megaspore functions and after undergoing three more divisions produces a mature embryo sac of the Polygonum type (Figs. 34–36) as in other members of this family (Dahlgren, 1916).

As a result of the enlargement of the embryo sac the nucellar epidermis is completely crushed, with the result that the wall of the embryo sac comes in direct contact with the innermost layer of the inner integument, which

now functions as the endothelium (Figs. 36 and 37). It surrounds the embryo sac on either side, from base to the apex. At a later stage its cells become vacuolated (Fig. 40). The endothelial cells are rigid and tannin-filled as in *Centunculus*, *Hottonia* and *Samolus* and they persist in the mature seed.

The embryo sac is elongated and broad in the middle. The pear-shaped egg is situated in between the long synerygids (Fig. 36). The nucleus of the synergid is situated in the centre of the cell. The polar nuclei meet nearer the egg apparatus. The antipodals are organised as cells (Fig. 36) and they are ephemeral as in other genera. In *Lysimachia vulgaris* (Dahlgren, 1916) the antipodals enlarge and show characteristic basal vacuoles, thus simulating the synergids.

The pollen tube enters the embryo sac by destroying one of the synergids. Double fertilisation has been observed (Fig. 37). Sometimes, the pollen tube enters the embryo sac without destroying the synergids. In such cases the synergids persist for some time becoming larger and more prominently vacuolated than usual (Figs. 38, 39).

ENDOSPERM

The primary endosperm nucleus lies in the centre of the embryo sac and divides (Fig. 40) forming two free nuclei. The orientation of the spindle is longitudinal as in *Primula officinalis* (Dahlgren, 1916). Both the free nuclei divide simultaneously (Fig. 41) and by further divisions a free nuclear endosperm is formed (Figs. 42 and 43). About the thirty-two or sixty-four free nucleate stage, wall formation sets in, and the endosperm thus becomes cellular. The cells of the endosperm store oils and proteins (Fig. 68). There are eight to sixteen or even thirty-two free endosperm nuclei at the fertilised egg stage. Sometimes only sixteen nuclei are found at the four-celled proembryonal stage (Fig. 43). Dahlgren (1916) reported the presence of about thousand free nuclei during the first division of the fertilised egg in *Primula officinalis*; and in *Hottonia palustris* he counted about two hundred free endosperm nuclei at the two-celled proembrycnal stage.

EMBRYOGENY

The fertilised egg (Fig. 44) divides by a transverse wall forming an upper cell *ca* and a lower cell *cb* (Fig. 45). Usually, cell *cb* divides earlier than cell *ca* (Fig. 46); sometimes the latter divides earlier (Fig. 48) producing cells *cc* and *cd* (Figs. 49 and 50). In either case a linear proembryo of four cells is formed (Fig. 50). Next, *cc* divides by a vertical wall (Figs. 51 and 52) and by one more division at right angles to the first, forms a group of four cells *q* (Fig. 54). Meanwhile *cd* divides transversely producing *m* and

ci (Figs. 51–54). Sometimes *cc* and *cd* divide simultaneously (Figs. 51 and 52). Cell *m* now divides vertically (Fig. 55) and *ci* transversely forming *n* and *n'* (Figs. 57 and 58). The four cells of tier *q* divide transversely and form tiers *l* and *l'* (Fig. 59). Cells *l*, *l'*, *m* and *n* take part in the formation of the different regions of the embryo. Cells *n'* and derivatives of the cell *cb* form the suspensor.

By anticlinal divisions in tiers *l*, *l'* and *m* the dermatogen is delimited from an inner group of cells (Fig. 60). By further vertical, followed by periclinal divisions in the inner cells of tiers *l* and *l'*, the periblem and plerome are organised. In the two inner cells of tier *m* (Fig. 60) oblique walls are laid down; they are followed by periclinal walls, resulting in the formation of two cells (Figs. 63 and 64) and these now complete the periblem in the upper region of the embryo. At the same time the single cell in tier *n* (Fig. 60) divides vertically (Figs. 61–63) and by one more vertical division at right angles to the first forms a group of four cells arranged in a tier. These soon divide transversely (Fig. 65) and along with similar divisions in the dermatogen cells of tier *m* in that region, take part in the organisation of the root tip and cap at the upper region of the embryo (Fig. 66). The mature embryo is erect and dicotyledonous (Fig. 67). The mode of embryo development closely corresponds with that of *Samolus Valerandi* (Souéges, 1937).

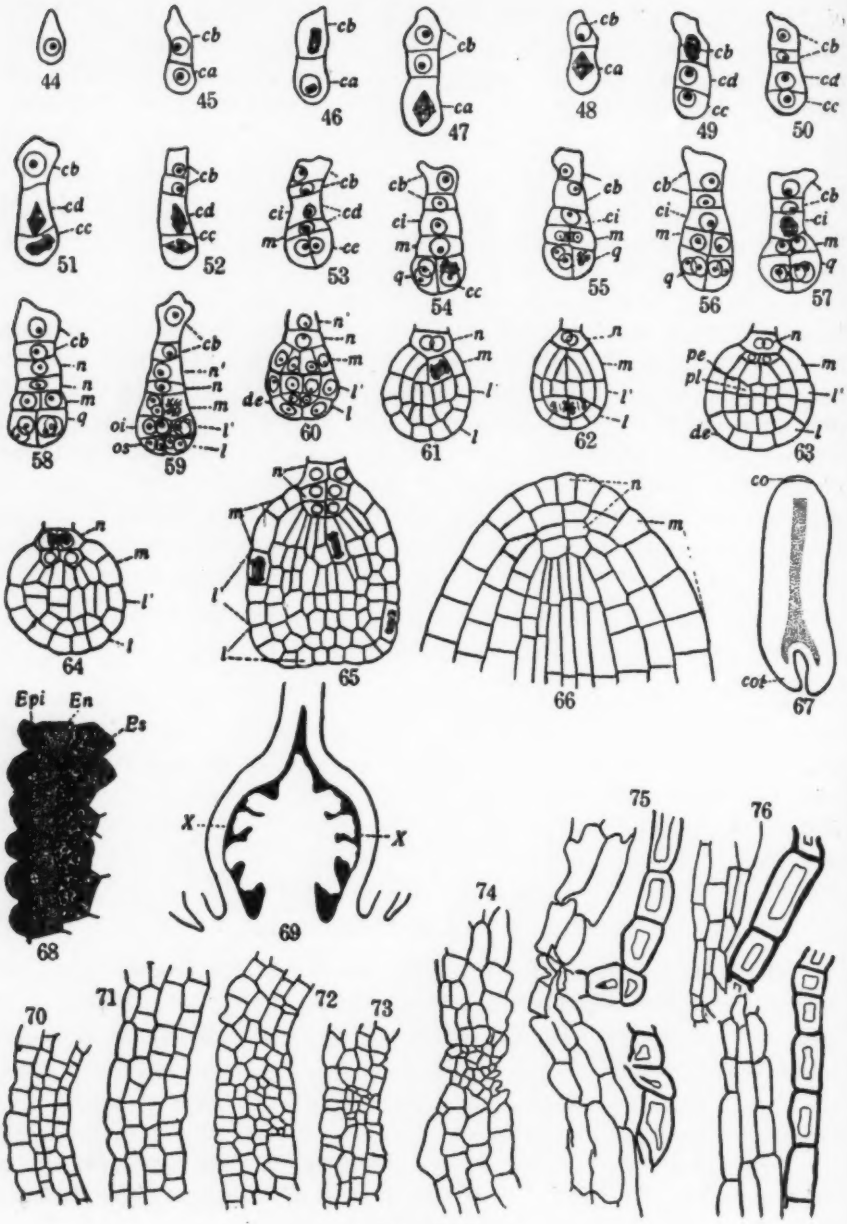
Seed Coat

In the mature seed only the epidermis and the endothelium persist and the remaining cell layers degenerate. The epidermal cells present an irregular outline and are filled with tannin. The large endothelial cells are also filled with rich tannin and enclose crystals of calcium oxalate (Fig. 68), as in *Samolus Valerandi* (Dahlgren, 1916). The tannin filled cells of the epidermis and endothelium are hard and rigid like those of *Samolus Valerandi*, *Centunculus minimus* and *Hottonia palustris* (Dahlgren, 1916).

Histology of the Mature Fruit

The mature fruit is an ovoid pyxidium and at the time of dehiscence shows two regions, an upper lid and a lower base. The zone of dehiscence appears as a circumcissile line, more or less in the middle of the fruit (marked X in Fig. 69).

In a longitudinal section the young ovary is globular in outline with several anatropous ovules borne on a globose free central placenta (Fig. 69). The young ovary wall is four layered and does not show the future line of dehiscence (Figs. 70 and 71) at this stage as in *Hyoscyamus niger*, *Portulaca grandiflora* and species of *Plantago* (Rethke, 1946).


44
cb
ca
45
46
47
48
cd
cc
49
50
51
52
ci
m
53
q
54
55
56
57
n
n'
l
l'
58
oi
os
59
de
60
61
62
pe
pl
63
64
65
66
co
cot
67
Epi
En
Es
68
X
69
70
71
72
73
74
75
76

FIGS. 44–76

FIGS. 44–67.—Figs. 44–67. Stages in the development of embryo. All, ×450. Except Fig. 62, ×485; and Fig. 63, ×215. *ca* = Terminal cell of two-celled proembryo. *cb.* = Basal cell of two-celled embryo. *cc.* and *cd.* = Daughter cells derived from *ca.* *m.* and *ci.* = Daughter cells derived from *cd.* *n.* and *n'.* = Daughter cells derived from *ci.* *q* = The two juxtaposed daughter cells of *cc.* *l.* and *l'.* = Lower and upper groups of cells in a two-tiered octant pro-embryo, which together are equivalent to *q.* *os.* = Superior octant. *oi.* = inferior octant. *de.* = dermatogen. *pe.* = periblem. *pl.* = plerome. *cot.* = cotyledon. *co.* = root cap. Fig. 68. Portion of the seed coat enlarged showing the epidermis (*Epi.*), endothelium (*En.*) with calcium oxalate crystals and the endosperm (*Es.*), ×485. Fig. 69. L.S. young ovary, ×100. Figs. 70–76. Stages showing the histology of pyxidium, ×450.

At the mature stage of the fruit the innermost layer of the ovary wall becomes elongated and prominently lignified (Figs. 75 and 76). At the region of dehiscence (marked X in Fig. 69) the thin-walled cells in the ovary wall become meristematic (Figs. 72–74). At a later stage these cells disorganise (Figs. 75 and 76), with the result that a portion of the ovary wall above this region comes out in the form of a lid, from the lower base (Fig. 76). The opening of the lid may also be brought about by the enlarging seeds which now fill the locule of the ovary completely. The histology of the pyxidium of *Anagallis pumila* resembles species of *Plantago* described by Rethke (1946) except that in *Plantago* the outermost layer of the ovary wall becomes lignified (Rethke, 1946).

SUMMARY

Glands are present on the floral parts and their development has been described in detail.

The tapetal cells of the anther are uninucleate and are of the glandular type. The pollen grains are two-celled; sometimes they germinate *in situ* within the anther locule.

The bitegmic tenuinucellate anatropous ovules are borne on a globose free central placenta. The innermost layer of the inner integument forms the endothelium. The development of the embryo sac follows the Polygonum type. Double fertilisation has been observed.

The endosperm is free nuclear but later becomes cellular. Its cells enclose proteinaceous and oily contents.

Development of the embryo has been studied. In the mature seed only the epidermis and the endothelium persist. The fruit is a pyxidium.

ACKNOWLEDGEMENTS

am deeply thankful to Prof. P. Maheshwari for valuable suggestions and for going through the manuscript; and to Prof. L. N. Rao and Dr. K. Subramanyam for guidance and valuable suggestions.

LITERATURE CITED

Dahlgren, K. V. O. .. "Zytologische und embrylogische Studien über die Reihen Primulales und Plumbaginales," *K. Svenska Vet.-Akaa. Handl.*, 1916, **56**, (4), 1–80.

Engler, A. and Prantl, K. .. "Die Natürlichen Pflanzenfamilien," **4**, *Teil. Abt.*, 1889, 1 and 2, 98–116.

Gamble, J. S. .. *Flora of the Presidency of Madras*, 1925, Vol. **2**, London.

Hooker, J. D. .. *Flora of British India*, 1879, London.

Rendle, A. B. .. *The Classification of Flowering Plants*, 1938, Vol. **2**, Cambridge.

Rethke, R. V. .. "The anatomy of circumcissile dehiscence," *Amer. Jour. Bot.*, 1946, **33**, 677–83.

Souèges, R. .. "Embryogénie des Primulacées. Développement de l'embryon chez le *Samolus Valerandi* L.," *C. R. Acad. Sc.*, Paris, 1937, **204**, 145.

894-52 Printed at the Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore.

FUNGI IMPERFECTI FROM MADRAS—I

By C. V. Subramanian

(*University Botany Laboratory, Madras*)

Received February 13, 1952

(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

It is proposed to describe in a series of papers the Fungi Imperfecti occurring in the Madras State, and this paper is the first of the series. Species recorded for the first time in the State are indicated by a single asterisk, and those recorded for the first time in India by two asterisks.

1. ***Torula herbarum* (Pers.) Link ex Fries, 1832, *Syst. Mycol.*, III, ii, p. 501.

Saccardo, *Syll. Fung.*, 4: 256, 1886; Mason, E. W., *Annotated Account of Fungi Received at the Imperial Mycological Institute*, List II (Fascicle 3-Special Part), 1941, pp. 113–16, ic.

Colonies black, powdery; mycelium scanty; hyphæ brownish, septate, 1–3 μ broad. Conidia produced in chains, which may be branched or unbranched, unbroken chains up to 100 μ long, arising from the swollen apex of a hypha or a lateral branch, or from a subglobose cell arising laterally from a hypha; conidia produced in acropetal succession, 4–10 μ broad, dark brown in colour when old, hyaline when young, and usually falling apart into short lengths, singly, in twos, in threes, in fours or fives, markedly or faintly echinulate. The older cells of fragments of conidial chains are often ruptured.

The following collections have been examined and include a few from the Travancore-Cochin State*: On dead leaf-sheath of *Cocos nucifera* L., Santhome, Madras, 14–2–1951, coll, C.V.S. (No. 48), Luz, Madras, 6–3–1951, coll. K.R. and C.V.S. (No. 134); on dead leaf rachis of *Cocos nucifera* L., Ernakulam (Travancore-Cochin), 7–9–1951, coll. C.V.S. (No. 456); on dead peduncles of *Areca catechu* L., Thottumugham, Alwaye (Travancore-Cochin), 7–9–1951, coll. C.V.S. (No. 482); on dead leaf-sheath of *Musa paradisiaca* L., Ernakulam, 6–9–1951, coll. C.V.S. (No. 467); on dead stems of *Acalypha wilkesiana* Muell. Arg., Univ. Bot. Lab. campus, Madras, 21–2–1951, coll.

* The following abbreviations are used to indicate the name of the collector K.R. = K. Ramakrishnan; C.V.S. = C. V. Subramanian. The numbers in brackets indicate accession numbers of the collections in Herb. M.U.B.L.

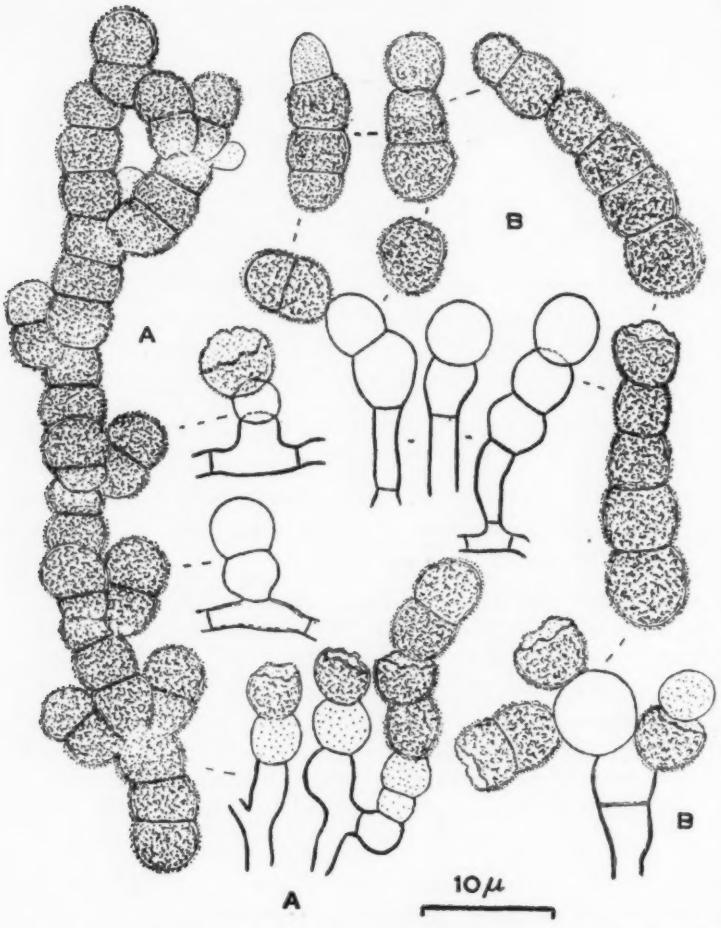


FIG. 1. *Torula herbarum*. A, from Herb. M.U.B.L. No. 578; B, from No. 499.

C.V.S. (No. 88); on dead stem of *Artocarpus integrifolia* L., Ernakulam, 7–9–1951, coll, C.V.S. (No. 499); on dead stems of *Cajanus cajan* (Linn.) Millsp., Univ. Bot. Lab. campus, Madras, 18–12–1951, coll. C.V.S. (No. 659); on dead stem of *Barleria* sp., St. Berchmann's College campus, Changanacherry (Travancore-Cochin), 5–10–1951, coll. K.R. (No. 579); on dead stem (? Crassulaceæ), Agr. Hort. Soc. Garden, Madras, 21–12–1951, coll. C.V.S. (No. 667); on dead stem (? Euphorbiaceæ), Agr. Hort. Soc. Garden, Madras, 21–12–1951, coll. C.V.S. (No. 668); on dead stem (Rubiaceæ), Ernakulam, 7–9–1951, coll. C.V.S. (No. 492); on dead stems, Univ.

Bot. Lab. campus, Madras, 12–8–1951, coll. C.V.S. (No. 282); 30–8–1951, coll. C.V.S. (No. 430), St. Berchmann's College campus, Changanacherry, 5–10–1951, coll. K.R. (No. 578).

2. ***Zygosporium oscheoides* Montagne, 1842, in *Ann. Sci. nat.*, 2 Ser., xvii, p. 121.

Saccardo, *Syll. Fung.*, 4: 329, 1886; Mason, E.W., *Annotated Account of Fungi Received at the Imperial Mycological Institute*, List. II (Fascicle 3-Special Part), 1941, p. 134–35, 139–43, ic; Hughes, S. J., *Mycol. Pap. C.M.I.*, 44, pp. 2–5, ic, 1951.

Repent hyphæ sub-hyaline to brown, 2–3 μ thick. Falciphores simple, erect, broader at the base and tapering upwards, concolorous with the brown hyphæ except for the apical cell, 2–3 septate, 35–65 μ long, 3–4 μ broad at the base; apical cell of the falciphore hyaline to subhyaline, narrowly clavate at maturity and falling off. Falces normally arising as lateral branches at right angles to the falciphores, one from the base of each falciphore; stipe usually obconic, one-celled, concolorous, 3–7 × 3–5 μ; prophialide brown to black, strongly curved, 9–15 μ along its greatest length, 4–10 μ across its greatest width; phialides subhyaline, obpyriform with a tapering neck when mature, 4–7 × 3–7 μ. Conidia hyaline, oval, smooth, 5–10 × 4–8 μ.

The following collections have been examined and includes one from the Travancore-Cochin State: On dead leaves (No. 84) and petioles (No. 86) of *Musa paradisiaca* L., Univ. Bot. Lab. campus, Madras, 20–2–1951, coll. C.V.S.; on dead leaf-sheath of *Musa paradisiaca* L., Luz, Mylapore, Madras, 6–3–1951, coll. K. R. and C. V. S. (No. 129); on rotting leaves of *Odina wodier* Roxb., Univ. Bot. Lab. campus, Madras, 30–8–1951, coll. K.R. (No. 431), 28–9–1951, coll. K.R. (No. 528); on decaying leaves of *Ixora* sp., Ernakulam (Travancore-Cochin), 7–9–1951, coll. C. V. S. (No. 504).

3. *Beltrania indica* Subramanian sp. nov.

Coloniæ sepiaceo-brunneæ, effusæ, velutinæ. Setæ fusce brunneæ, rectæ, erectæ, distanter septatæ, crassis parietibus præditæ, 120–325 μ longæ, 3–5 μ latæ supra basim tumescentem, desinentes in apicem acutum atque pallidum. Conidiophori simplices, raro furcati in apice, erecti, recti vel curvati, usque septies septati, emergentes simul cum setis turmatim, 30–130 μ longi, 3–4 μ lati, brunnei colore ad basim, 4–7 μ lati ad apicem subhyalinum, qui est distincte denticulatus; "cellulæ separantes" hyalinæ, ovatæ, denticulatæ in utroque apice, 4–12 × 3–5 μ. Conidia bicornia, levia, sicca, unicellularia, pallide vel fusce brunnea, zona equatoriali pallidiori vel subhyalina ornata, 13–25 × 8–12 μ (mediet. 21 × 10) μ, rotundata et cicatrice

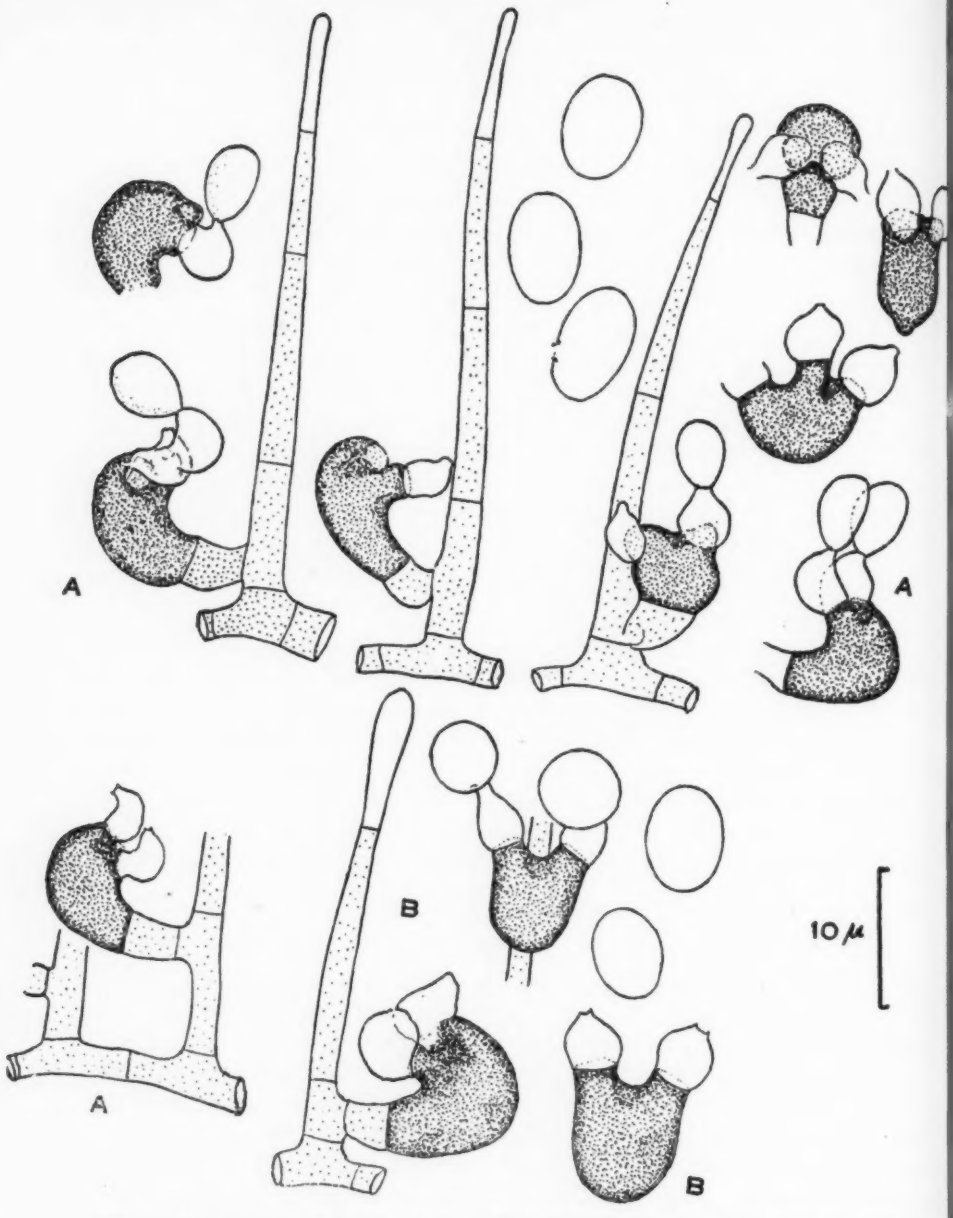


FIG. 2. *Zygosporium oscheoides*. A, from Herb. M.U.B.L. No. 528 ; B, from No. 504.

ornata vel denticulata ad basim, ornata unica seta longa, conica, unicellulata, hyalina vel subhyalina ad apicem, seta vero est 2–10 μ longa, circiter 2 μ lata

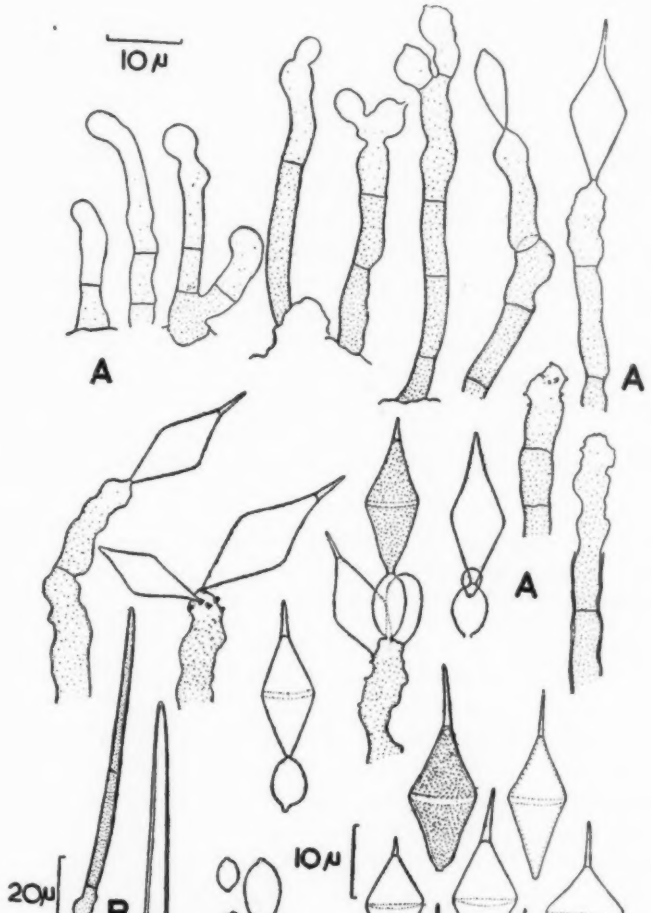


FIG. 3. *Beltrania indica* from Herb. M.U.B.L. No. 673. A, showing conidiophores and conidia and their development; B, setæ; C, "separating cells"; D, mature conidia.

ad basim. Conidia singula insident " cellulis separantibus," vel nulla " separante cellula " directe conidiophoro insident.

Habitat in fructibus emortuis *Cæsalpiniæ pulcherrimæ* Sw. (Leguminosæ, Madras, in campo Universitatis Botanices laboratorii, 4 januarii anni 1952) legit C.V.S., No. 673 (Typus); 28 Januarii 1952, legit C.V.S., No. 716.

Colonies coffee brown, effuse, velutinous. Setæ dark brown, straight, erect, distantly septate, thick-walled, 120–325 μ long, 3–5 μ broad just above the swollen base, tapering to an acute and paler apex. Conidiophores simple, rarely forked into two at the tip, erect, straight or bent, up to 7-septate, arising in groups along with setæ 30–130 μ long, 3–4 μ wide and brown in colour at the base, 4–7 μ wide at the subhyaline to hyaline apex which is markedly denticulate; " separating cells " hyaline, oval, denticulate at both ends, 4–12 × 3–5 μ. Conidia biconic, smooth, dry, unicellular, pale to dark brown, with a paler or subhyaline equatorial band, 13–25 × 8–12 (mean 21 × 10) μ, rounded with a scar or denticulate at the base, with a long, conical, one-celled, hyaline to subhyaline seta at the apex, 2–10 μ long and about 2 μ wide at the base. Conidia are borne singly on the " separating cells", or without a " separating cell " and borne directly on the conidiophore.

Habit: on dead pods of *Cæsalpinia pulcherrima* Sw. (Leguminosæ), Univ. Bot. Lab. campus, Madras, 4–1–1952, coll. C.V.S., No. 673 (Type); 28–1–1952, coll. C.V.S., No. 716.

Four species of *Beltrania* are known: *B. rhombica* Penzig (the type species), *B. querna* Harkness, *B. malaiensis* Wakefield, and *B. africana* Hughes (*see* Hughes, *Mycol. Pap. C.M.I.*, 47, 1951). In conidial measurements *B. indica* comes closest to *B. querna* but differs from it, and also from all the other species, in having much longer setæ and conidiophores, and smaller conidia. Following the authors cf *Beltrania querna*, *B. malaiensis* and *B. africana*, this fungus is placed in the genus *Beltrania*, although the type species *B. rhombica* is stated to have one- septate conidia.

4. ***Stachybotrys atra* Corda, 1837, in *Ic. Fung.* I, p. 21, f. 278 B.

Saccardo, *Syll. Fung.*, 4: 269. 1886; Bisby, G. R., *Trans. Brit. mycol. Soc.*, 26: 133–43. 1943; 28: 11–12. 1945.

Colonies black; mycelium subhyaline to hyaline, septate, 2–4 μ broad; phialophores 30–65 × 2–4 μ, brownish except at the base which is subhyaline, 1–2-septate, the apical cell of the phialophore paler in colour, often longer than the lower cells, with a slightly swollen tip on which are borne

phialides; phialides 5–8, subhyaline or faintly coloured, obovate to pyriform or clavate, 6–10 × 2–4 μ. Mature conidia opaque black, smooth, oval, 3–10 × 2–4 μ.

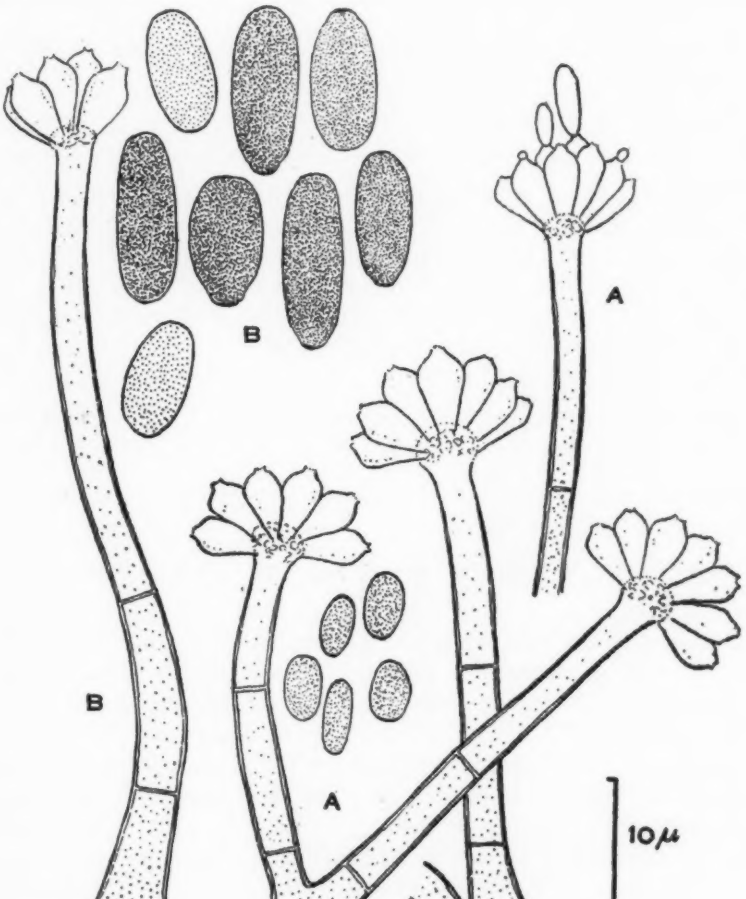


FIG. 4. *Stachybotrys* : A. *S. atra*, phialophores and conidia, from Herb. M.U.B.L No. 83 ; B, *S. pulchra*, phialophores and conidia, from Herb. M.U.B.L. No. 614.

The following collections have been examined and includes one from the Travancore-Cochin State; On dead leaf base of *Musa paradisiaca* L.,

Univ. Bot. Lab. Campus, Madras, 20–2–1951, coll. C.V.S. (No. 83); on dead leaf-sheath of *Musa paradisiaca* L., Ernakulam (Travancore-Cochin), 6–9–1951, coll. C.V.S. (No. 465); on paper, Univ. Bot. Lab. campus, Madras, 28–11–1951, coll. C.V.S. (No. 633).

5. ***Stachybotrys pulchra* Spegazzini, 1896, in *Rev. Agr. y. Veter. La Plata*, p. 248.

Saccardo, *Syll. Fung.*, 14: 1071. 1899; Bisby, G. R., *Trans. Brit. mycol. Soc.*, 26: 141. 1943 (as a possible synonym of *S. atra* Corda).

Colonies blackish; mycelium hyaline to subhyaline, 2–4 μ broad, septate; phialophores 50–110 $\times$ 2–4 μ, brownish except at the base which is subhyaline, 1–2-septate, the apical cell of the phialophore often longer than the lower ones, with a slightly swollen subhyaline tip on which are borne phialides; phialides up to 5, subhyaline, obovate to pyriform or clavate 11–17 $\times$ 2–4 μ; mature conidia greenish black to opaque black, smooth, oval, 8–19 $\times$ 4–7 (mean 15 $\times$ 5·8) μ.

Only one collection has been examined: on dead leaf of *Ficus bengalensis* L., Univ. Bot. Lab. campus, Madras, 6–11–1951, coll. C.V.S. (No. 614).

6. **Memnoniella echinata* (Riv.) Galloway, 1933, in *Trans. Brit. mycol. Soc.*, 18: 163–66, ic.

Bisby, G. R., *Trans. Brit. mycol. Soc.*, 26: 133–43; 1943; 28: 11–12, 1945.

Colonies deep black, velvetty to floccose, up to 10 mm. long and 5 mm. broad; mycelium composed of hyaline, septate hyphæ, 2–3 μ thick; conidiophores erect, hyaline at the base and black to greenish black above, 2–3-septate, 60–170 $\times$ 2–5 μ, slightly swollen and 4–5 μ broad at the tip which is hyaline; phialides hyaline, with characteristic shape, 6–12 $\times$ 3–7 μ, usually about seven in number, and arising from the swollen tip of the conidiophore; conidia deep black, globose to squarish, echinulate, 5–9 μ in diameter, produced basipetally from the tip of the phialides in chains, chains up to 160 μ long and with over 50 conidia, often breaking up into short chains of two or three conidia.

The following collections have been examined: On decaying palm frond, near Senate House, Chepauk, Madras, 13–2–1951, coll. K.R. and C.V.S. (No. 41); on dead petioles of *Musa paradisiaca* L., Univ. Bot. Lab. campus, Madras, 20–2–1951, coll. C.V.S. (No. 87); on dead leaf-sheath of *Musa paradisiaca* L., Luz, Mylapore, Madras, 6–3–1951, coll. K.R. and C.V.S. (No. 130); on dead stems of *Euphorbia tirucalli* L., Agr. Hort.

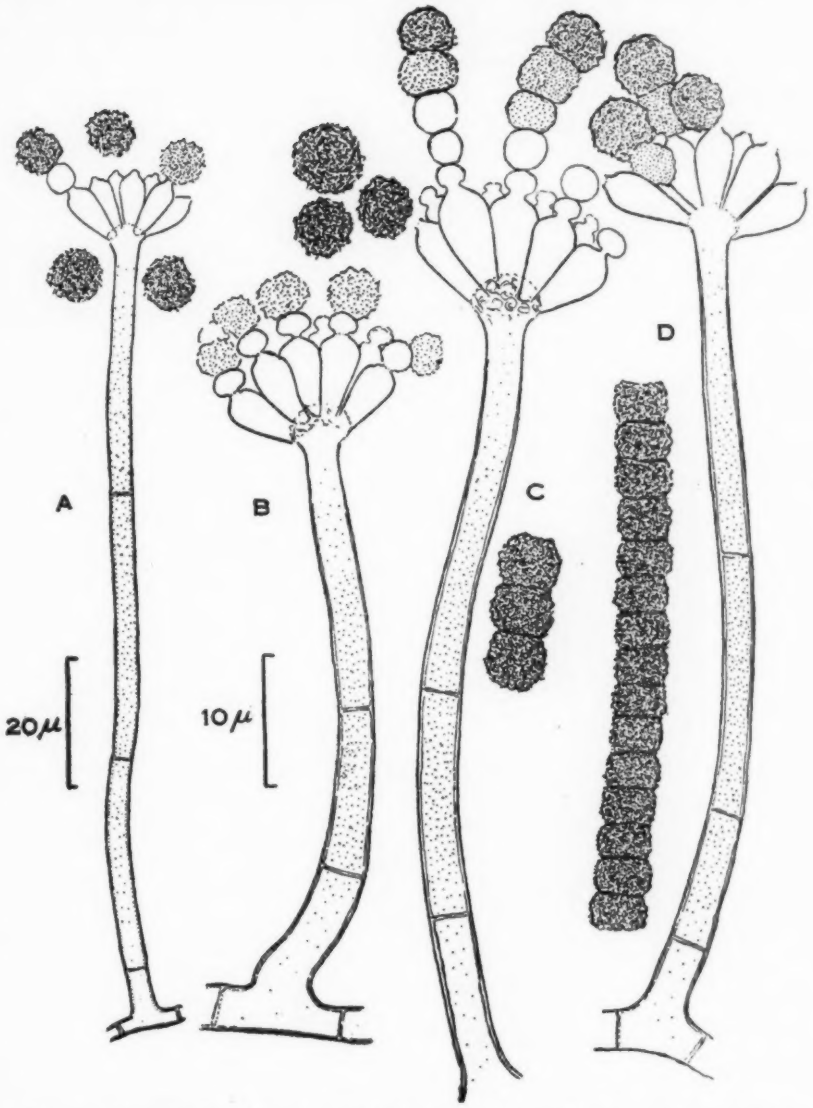


FIG. 5. *Memnoniella echinata*, phialophores and conidia from four collections: A, from Herb. M.U.B.L. No. 130; B, from No. 99; C, from No. 656; D, from No. 494.

Soc. Garden, Madras, 25-2-1951, coll. K.R. and C.V.S. (No. 99); on decaying spathe of *Areca catechu* L. (the spathe came from Mysore as pack-

ing material for arecanuts), 14–9–1951, coll. K.R. and C.V.S. (No. 494); on dead stems of *Cyamopsis tetragonoloba* Taub., Univ. Bot. Lab. campus, Madras, 25–10–1951, coll. C.V.S. (No. 605); on dead seed of *Thevetia neriifolia* Juss., Univ. Bot. Lab. campus, Madras, 17–12–1951, coll. C.V.S. (No. 656); on dead stem, Univ. Bot. Lab. campus, Madras, 12–8–1951, coll. C.V.S. (No. 283).

7. ***Corynespora cassiicola* (Berk. and Curt.) Wei, 1950, in *Mycol. Pap. C.M.I.*, 34, p. 5, ic.

Colonies brownish in colour, up to a centimetre or more in length and 5 mm. wide; mycelium deep brown, septate; conidiophores arising per-

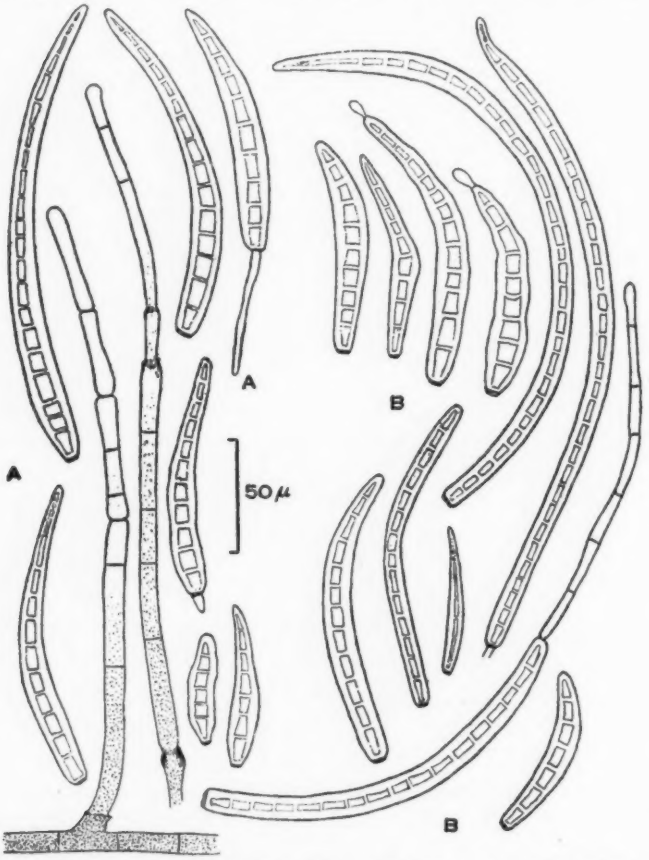


FIG. 6. *Corynespora cassiicola* from two collections : A, from Herb. M.U.B.L. No. 474 ; B, from No. 584.

pendicularly from the repent hyphæ, sometimes swollen at the base, of variable length (50–400 μ), septate, concolorous with the hyphæ, 3–9 μ wide; conidia borne singly at the tip, usually connected by a hyaline isthmus to the conidiophore, mostly cylindrical or sometimes obclavate, straight or slightly or markedly curved, tapering slightly or markedly towards the apex, subhyaline, up to 30-pseudoseptate, 35–280 × 7–18 μ, germinating by polar germ tubes.

No conidial chains have been seen. The conidiophores proliferate terminally through the scar of the fallen conidia or sometimes through the injured tip of the conidiophore. The proliferating joints are variable in length (30–190 μ) and are lighter in colour towards the apex.

Four collections have been examined of which two are from Travancore-Cochin State: On dead stem of *Cassia* sp., Kolathuraar, Chingleput District (Madras State), 2–9–1951, coll. K.R. (No. 439); on rotting leaves of *Carica papaya* L., Chingavanam (Travancore-Cochin), 8–10–1951, coll. K.R. (No. 584); on dead stem, Ernakulam (Travancore-Cochin), 7–9–1951, coll. C.V.S. (No. 474); Kolathuraar, 2–9–1951, coll. C.V.S. (No. 508).

I am grateful to Prof. T. S. Sadasivan for reading the manuscript; to Sri. K. Ramakrishnan for some of the collections; and to the Rev. Dr. H. Santapau for translating into Latin the diagnosis of *Beltrania indica*.

SOIL CONDITIONS AND ROOT DISEASES

V. Symptomatology of Wilted Cotton and Red Gram

By G. Satyanarayana and R. Kalyanasundaram

(*University Botany Laboratory, Madras*)

Received February 25, 1952

(Communicated by Professor T. S. Sadasivan, F.A.SC.)

Introduction

Although wilts of cotton (*Gossypium* spp.) and red gram (*Cajanus cajan*) have been studied in this country and elsewhere (Butler, 1906; Fulton, 1907; Dastur, 1924, 1926 and 1931; Fikry, 1932; Kulkarni, 1934; Subramanian, 1950; and Sarojini, 1950) produced by the soil-borne fungi *Fusarium vasinfectum* and *Fusarium udum*, no serious attempt has been made to precisely indicate wilt symptoms as seen in the aerial parts of the affected plants. Both wilts are known to be vascular in their mode of attack, the progress of wilt being conditioned to some extent, by a toxemic state of the plant.

In recent years much interest has centred round the discovery of antibiotics being formed in soils *in situ* and absorbed by plants detectable in the guttation fluid indicating systemic nature of fungal toxins (Waksman, 1947; Brian, *et al.*, 1951).

The following observations were made in this laboratory during the conduct of experiments on several aspects of the mechanism of wilts in plants and are of considerable fundamental and practical importance in detecting soil-borne fungal wilts of these two crop plants and are, therefore, described here.

Materials and Methods

Plants were grown in sterilised garden soil contained in earthenware pots. Two varieties of cotton, *Gossypium arboreum*, commonly called the Karunganni (K. 2) strain (Madras State) and the Malvi (M. 9) strain (Bombay State), and only one variety of red gram, *Cajanus cajan* (susceptible variety No. 1723 obtained from Kovilpatti) were used. Isolates of *Fusarium vasinfectum* (obtained from Centraalbureau voor Schimmelcultures, Baarn, Holland) and *Fusarium udum* (isolated from the rhizosphere of wilting red gram plant, grown in this Laboratory garden by Mr. V. Agnihothrudu) grown in soil oat (9 : 1) (Padwick, 1935) for a period of three weeks were the sources of inocula. Photographs were taken with " Contax " camera

(F 2; 5 cm. lens) using Ilford F.P. 3, 35 mm. film, the filter used being tricolour red (filter factor 5–6).

Experimental

Delinted seeds of cotton and surface sterilised seeds of red gram were sown separately in pots, the soils being previously inoculated (3 days) with the pathogens *F. vasinfectum* and *F. udum* respectively. The uninoculated pots of both the series served as control.

Germination was complete by the fourth day in the case of cotton and by the fifth day in the case of red gram. The first external symptom of wilt in the case of cotton was the yellowing of the veins of cotyledonary leaves (Plate II, Figs. 1 and 4) and also second leaves (Plate II, Fig. 1) and in the case of M. 9 and K. 2 varieties the first wilt symptom begins to appear on the seventh and thirteenth days respectively after germination. The intensity of yellow colour of the veins increased in about 24 hours and the leaves showed general dechlorophyllation and assumed a drooping condition. The time interval between the first sign of vein clearing (Plate II, Fig. 4) to the death of the plant was three days. In general, the severity of wilt symptom resulting in a quick death of the plants was greater in cotton than in red gram.

The external symptom of wilt in the case of red gram was general dechlorophyllation of all the leaves (Plate II, Fig. 5) except the cotyledonary leaves, which are not seen to develop above soil level, since the type of germination is hypogeal as opposed to the epigeal germination of cotton. In red gram, from the initial dechlorophyllation complete wilting was encountered in six or seven days, the first typical symptom being observable on or after the eighteenth day from the date of germination. The wilting of red gram plants in *F. udum* infected soils was more gradual when compared to the abrupt wilt production in the case of cotton in *F. vasinfectum* infected soils.

Discussion

Symptomatology of plants as a diagnostic method of soil-borne fungal root diseases, particularly in the case of wilts, has not kept pace with causative agents like viruses and deficiency diseases. This is largely because many of the symptoms that appear in plants following the entry of fungal wilt organisms through roots, result in either too sudden a yellowing followed closely by a drying up of the leaves, and indeed all the aerial parts, or there is an incipient derangement of normal metabolism not so easily discernible to the unaided eye. Nevertheless, considerable changes in the chlorophyll pigmentation resulting in what is called as " yellowing " have been reported

in most wilt diseases (Keyworth, 1938; Foster, 1946). We have presented in this paper photographs of the leaves of two wilted plants artificially inoculated with two distinct species of *Fusarium*. In the case of *F. vasinfectum* on cotton (Plate II, Figs. 1 and 4) there is a well-defined vein clearing in the cotyledonary leaves and less pronounced vein clearing in the second leaf (Plate II, Fig. 1) and this is clearly seen in the photograph although it is not easily noticeable to the naked eye. The other symptoms produced are more of the type of yellowing clearly seen by photographing on panchromatic films with tricolour red filter (Plate II, Fig. 5). Indeed, the yellowing is apparent in photographs even in the cases of cotton seedlings commonly termed 'disease escapes' (Plate II, Fig. 3) in infected soils although this dechlorophyllation is not perceptible to the eye as a clear symptom of incipient disease production. One of us (R. K.) has recently shown that the typically yellowed leaves of the seedlings of cotton and red gram following infection by *F. vasinfectum* and *F. udum* respectively indicate a deranged ascorbic acid build up over their usual undiseased counterparts and also register an increase in reducing sugar (glucose) content (these results will be discussed in full elsewhere). It would suffice for the subject under consideration here to state that both wilts discussed are vascular in origin (Plate II, Figs. 6 and 7) although symptoms of wilt in cotton appear sooner than in red gram. There is every indication of toxin movement in *F. vasinfectum* infected cotton resulting in a more apparent vein clearing symptom than in *F. udum* infected red gram which shows a gradual yellowing of the leaves and no vein clearing probably due to a mechanical plugging of the vessels resulting in a general loss of chlorophyll although the possibility of a toxemic condition cannot be completely overruled. Earlier work in this laboratory (Yogeswari, 1948; Sarojini, 1950; and Sulochana, 1952) indicated very distinct growth response by *F. vasinfectum* and *F. udum* in the presence of trace elements. This enables us to hypothesise the possible role of these trace elements in wilts in that the invading fungus may deprive the plant during their progress in the root system some of the essential chlorophyll forming elements (Hewitt, *et al.*, 1950). It may then follow that the dechlorophylled condition in the red gram without any vein clearing may be due to a certain amount of local deficiency of heavy metals.

SUMMARY

1. Wilt symptoms in cotton and red gram caused by the soil-borne pathogens *F. vasinfectum* and *F. udum* as seen by the naked eye and clearly made out by photographing with tricolour red filter on panchromatic film are recorded.

2. Wilt symptoms in cotton when sown in *F. vasinfectum* infected soils appear on the seventh day in variety M. 9 and thirteenth day in variety K. 2 after germination of the seeds as very distinct vein clearing discernible to the eye in cotyledonary leaves. Later, the first leaves also show vein clearing not clear to the eye but detectable by photographing with tricolour red filter.

3. Wilt symptoms in red gram by *F. udum* do not culminate in vein clearing as in cotton although there is a general and well-marked dechlorophyllation presenting almost a toxemic condition. This is first seen on the eighteenth day after germination.

4. The possibility of these vascular wilts interfering with the normal uptake of some of the essential chlorophyll forming heavy metals is discussed.

Acknowledgments

The authors are greatly indebted to Professor T. S. Sadasivan for guidance. We wish to acknowledge our gratitude to Dr. C. V. Subramanian and Mr. C. S. Venkatram for permission to use two photographs (Plate II, Figs. 5 and 6). We thank the Madras University for the award of studentships.

References

Brian, P. W., Wright, J. M., Stubbs, J. and Way, A. M. "Uptake of antibiotic metabolites of soil micro-organisms by plants," *Nature*, 1951, **167**, 347–49.

Butler, E. J. .. "The wilt disease of pigeon-pea and pepper," *Agric. J. India*, 1906, **1**, 25.

Dastur, J. F. .. "A preliminary account of the investigation of cotton wilt in Central Provinces and Berar," *Ibid.*, 1924, **19**,251–60.

———— .. "Cotton Wilt," *Mem. Dept. Agric. India* (Bot. Ser.), 1931, **17**, 29–75.

Fikry, A. .. "Investigations on the wilt disease of cotton (Egyptian) caused by various species of *Fusarium*," *Ann. Bot. Lond.*, 1932, **46**, 29–71.

Foster, R. E. .. "The first symptom of tomato wilt : Clearing of the ultimate veinlets in the leaf," *Phytopathology*, 1946, **36**, 691–98.

Fulton, H. R. .. "Cotton Wilt," *La Expt. Sta. Bull.*, 1907, **96**.

Hewitt, E. J., Agarwala, S. C., and Jones, E. W. "Effect of molybdenum status on the ascorbic acid content of plants in sand culture," *Nature*, 1950, **166**, 1119.

Keyworth, W. .. "*Verticillium* wilt of Hops," *Rep. E. Malling Res. Sta.*, 1938.

Kulkarni, G. S. .. "Studies in the wilt disease of cotton in the Bombay Presidency," *Indian J. agric. Sci.*, 1934, **4**, 976–1048.

Padwick, G. .. "Influence of wilt and cultivated plants on the multiplication, survival and spread of cereal foot-rotting fungi in ihe soil," *Canad. J. Res.*, C., 1935, **12**, 575–89.

Sarojini, T. S. .. "Soil conditions and root diseases. II. *F. udum* disease of red gram (*Cajanus cajan*)," *Proc. Indian Acad. Sci.*, 1950, **33 B**, 1–67.

Subramanian, C. V. .. "Soil conditions and wilt diseases in plants with special reference to *F. vasinfectum* on cotton," *Ibid.*, 1950, **31 B**, 67–101.

Sulochana, C. B. .. "Soil conditions and root diseases. III. With special reference to colonisation and survival of soil Fusaria in soils treated with micro-elements," *Ibid.*, 1952, **35 B**, 209–213.

Waksman, S. A. .. "*Microbial Antagonism and Antibiotic Substances*. The Commonwealth Fund, New York, 2nd Edn., 1947.

Yogeswari, L. "Trace element nutrition of fungi. I. The effect of Boron, Zinc and Manganese on *Fusarium* species," *Proc. Indian Acad. Sci.*, 1948, **28 B**, 177-201.

EXPLANATION OF PLATE

PLATE II

FIG. 1. Protograph of *F. vasinfectum* infected cotton (*Gossypium arboreum* variety K. 2) showing distinct vein clearing in cotyledonary and first leaf. $\times Ca.\ 1\frac{1}{2}$.

FIG. 2. Photograph of control cotton plant (uninfected) of the same age and variety as above with no vein clearing symptoms. $\times Ca.\ 1\frac{1}{2}$.

FIG. 3. (*a*) Healthy control cotton (variety K. 2) seedling.

(*b*) Wilted plant completely shrivelled.

(*c*) 'Escape' plant in wilt sick soil showing slight dechlorophyllation and tendency of wilt. $\times Ca.\ \frac{3}{4}$.

FIG. 4. (*a*) Cotton seedling (variety K. 2) in infected soil showing distinct early vein clearing in the cotyledonary stage 13 days after germination.

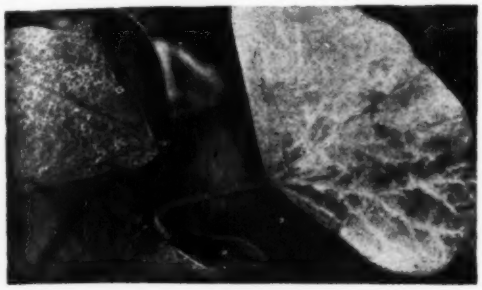
(*b*) Apparently healthy seedling ("escape") in the same pot $\times Ca.\ \frac{3}{4}$.

FIG. 5. (*a*) Completely dechlorophylled diseased red gram (*Cajanus cajan* susceptible variety) plant with no vein clearing as seen in cotton. Tips of leaflets are markedly paler than the rest of the lamina.

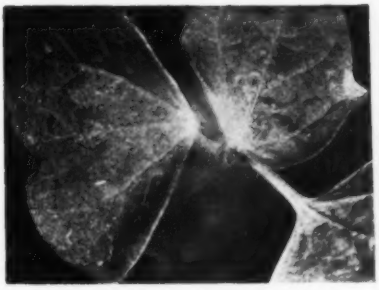
(*b*) Healthy control with characteristic dark chlorophyll pigmentation. $\times Ca.\ \frac{3}{4}$.

FIG. 6. T.S. of cotton root showing vascular invasion of *Fusarium vasinfectum*. $\times 600$.

FIG. 7. L.S. of red gram root showing vascular invasion of *Fusarium udum*. $\times 350$.

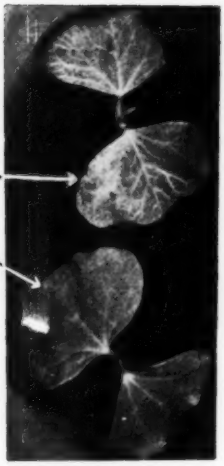


1



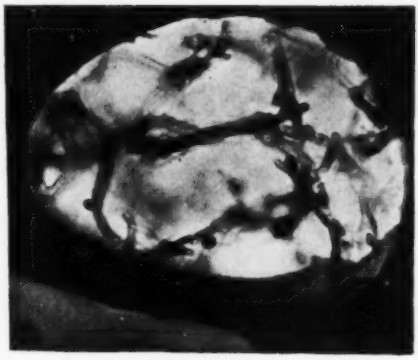
2

3

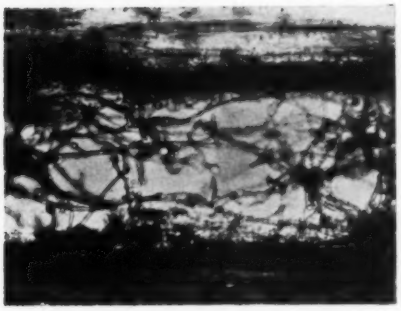


4

5



6



7

STUDIES IN THE ORDER PARIETALES

III. Vascular Anatomy of the Flower of *Carica papaya* L., with Special Reference to the Structure of the Gynæceum*

BY SHANTI DEVI, M.SC.

(*Botany Department, Meerut College, Meerut*)

Received February 14, 1952

(Communicated by Dr. V. Puri, F.A.SC.)

INTRODUCTION

CARICACEÆ is a small family consisting of four genera of which *Carica* is the most important with about 40 species in tropical and sub-tropical regions. The systematic position of the family has long remained doubtful. Usteri is reported (Worsdell, 1916) to have placed it near Euphorbiaceæ. Van Tieghem (1918) regarded it as allied to the Cucurbitaceæ. This view has been upheld by Hutchinson (1926). Hooker (1879) assigned it to the Passifloraceæ as an anomalous tribe, a position which has been followed up recently by Gundersen (1950). Rendle (1925), Engler and Prantl (1926), Warming and Potter (1932) and Wettstein (1935) have described it as a separate family related to the Passifloraceæ. General consensus of opinion, therefore, seems to be that the family is related to the Passifloraceæ and perhaps to the Cucurbitaceæ. Both the Passifloraceæ and Cucurbitaceæ have been studied in detail in this laboratory and some interesting structural peculiarities of the gynæceum have been brought to light (Puri, 1947 and 1951). It was with the idea of determining whether such peculiarities also exist in the Caricaceæ that Dr. V. Puri suggested to me to work out the floral anatomy of this family. A study of *Carica papaya* which alone is available here, reveals that the structure of the gynæceum is quite peculiar and hence I am publishing my results in the present form. Attempts are, however, being continued to procure alcohol preserved material of other species and genera from South America and elsewhere.

MATERIAL AND METHODS

Buds and flowers in different stages of development were collected at Meerut and preserved in F.A.A., serial microtome sections, 10μ–16μ thick were cut and stained with crystal-violet and erythrosin.

* The last paper of this series is: Puri, V. (1939), "Studies in the Order Parietales. II. A Contribution to the Morphology of *Garcinia livingstonii* T. Anders," *Proc. Ind. Acad. Sci.*, 9, 74-86.

OBSERVATIONS

External Morphology.—C. papaya is perhaps the best known species of the family. It is cultivated throughout the tropical and sub-tropical regions for its large edible fruits. The plant is up to 20 feet high, branched in old stages and bears a crown of long-petioled, palmate leaves on top. It may be monœcious or diœcious and flowering continues almost all the year round. The monœcious plants observed by the author bore alternate zones of staminate and pistillate flowers, the latter occurring below the former. The two types of flowers appear similar until the development of ovary in the pistillate flowers. In the diœcious cases the staminate flowers are smaller than the pistillate ones.

The staminate flowers from a diœcious and of a monœcious plant differ somewhat in some minor details. In the former case they are small, less leathery, pedicellate and have a very small yellowish bract, with pointed apex, while in the latter they are large, leathery, sub-sessile with a large leaf-like green bract. Higher up on the pedicel there are two bracteoles right and left of the bract. Each of these bears a more or less developed inflorescence in its axil. The five minute sepals are gamosepalous and alternate with the five petals which are also fused into a long (diœcious) or short (monœcious) corolla tube. The free ends of the petals have twisted æstivation. Alternating with the petals and occurring in two whorls of five each are ten stamens with short hairy filaments. The pistillode is better developed in flower from monœcious plants than in those from diœcious plants.

The pistillate flowers are pedicellate and bracteate. A little higher up there are two small bracteoles right and left of the bract and each having an inflorescence in its axil. The flowers are pentamerous, hypogynous and fleshy. The calyx resembles that of the staminate flower. But it is interesting to note that the corolla here is polypetalous. The petals are large and much twisted in bud. The ovary is sessile, fleshy and unilocular with a large number of anatropous ovules on five large placentæ. The styles are five free with dichotomously divided stigma which are much convoluted in young bud.

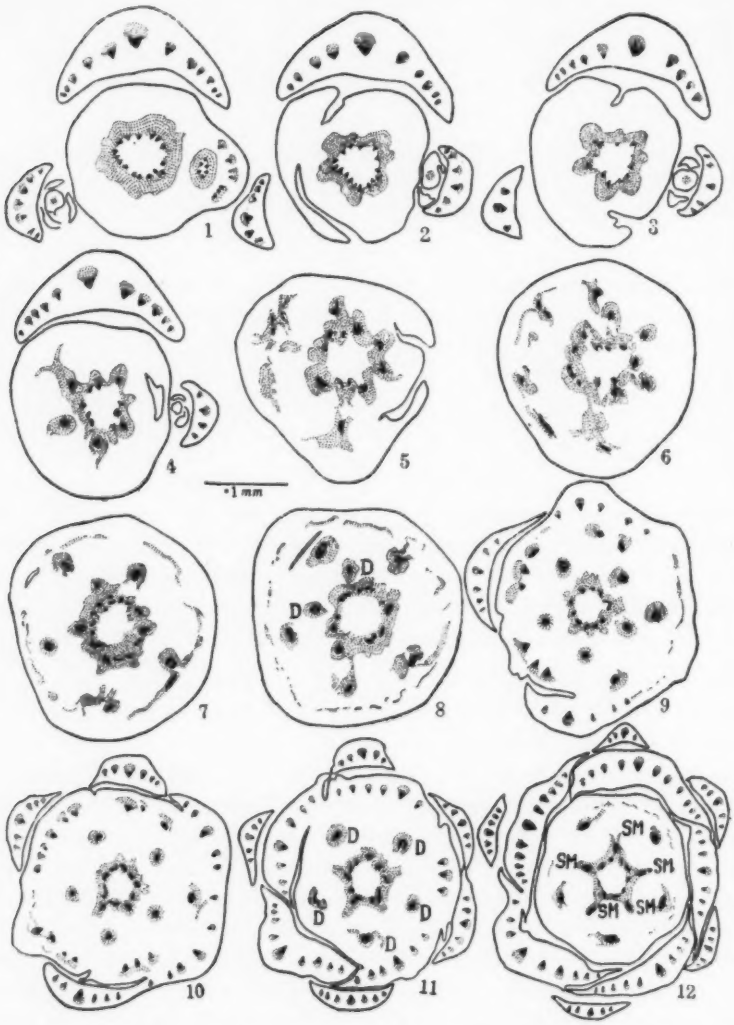
The hermaphrodite flowers occurring terminally on some twigs of some short-statured staminate plants have also been observed. Here, all the ten fertile stamens occur in one whorl and ovary is narrower and longer than that in the pistillate flower. The ovules also are fewer in number. Fusion of the petals continues until the stamens separate from the corolla obes for about one-fourth the length of the corolla.

Vascular anatomy of the flower.—The pedicel in the pistillate flower contains a dissected siphonostele of 10–16, unequal, endarch, vascular bundles, separated from one another by more or less broad parenchymatous strips. A small vascular bundle is given off from the receptacular stele to a bract. This bundle divides twice radially in the cortex of the receptacle to form four bundles of which the two median ones do not divide further, while the two lateral ones divide and subdivide forming in all about 12–15 bundles. The region containing these bundles soon separates off as a bract (Fig. 1).

Soon after, five traces separate from the central stele. All of these branch to form an irregular ring of vascular tissue in transverse section in the peripheral region of the receptacular cortex (Figs. 2–8). Some of these branches occurring on sepal radii diverge further out and enter the regions which will soon be marked off as sepals. Every sepal thus receives 2–3 or more branches which divide further. Out of these the central midrib bundle is the most prominent (Figs. 9–10).

After the sepal vascular supply has been marked off, the five parent bundles in the cortex migrate a little side ways and occupy the would-be midrib positions of petals. Every one of these gives off branches on either side and forms an arc of vascular tissue in transverse section. This enters the single petal which differentiates at this radius (Figs. 9–11).

Even before the separation of sepals and petals from the receptacle, the dorsal bundles of the five carpels begin to diverge. They are prominent, concentric bundles which generally occur on the same radii as the sepal midribs and alternate with the petals. Sometimes, however. this alternation of parts is not rigidly maintained. May be that complete suppression of the androœcium causes some disturbance. Every carpellary dorsal on reaching its destination gives off some branches laterally which extend into the carpellary wall. Five more bundles are marked off from the central stele on alternate radii but they separate off at a higher level (Figs. 12, 13). There is little doubt that they are secondary marginal strands, formed of secondary marginals, but in the following description they will be merely referred to as SM. When these are preparing to separate another set of five traces is given off on the radii on which dorsals had separated earlier (Fig. 13). These latter bundles set off rather quickly and immediately divide into two branches, each of which arranges itself on either side of the developing locule. They further split up into smaller branches which get consumed in supplying some of the first formed ovules (Figs. 13, 14). Whenever xylem elements are noticeable in these bundles they appear to occupy a more or



FIGS. 1–12. Diagrams of transverse section of a pistillate flower from base upward. Fig. 1. Shows a bract on the abaxial side and two bracteoles on either side of it. Figs. 2–5. Note the ridges on the pedicel being marked off. Bundles for sepals and petals are diverging from the receptacular stele. Figs. 6–7. The five bundles set off give off branches for sepals. Figs. 8–9. The remainders of the five parent bundles constitute the vascular supply of the petals. Note also the five carpellary dorsals coming out. Figs. 10–12. Sepals and petals are being marked off. Carpellary dorsals and secondary marginal strainds can also be seen.

less central position. While the large dorsal bundles are clearly endarch, particularly in the upper part.

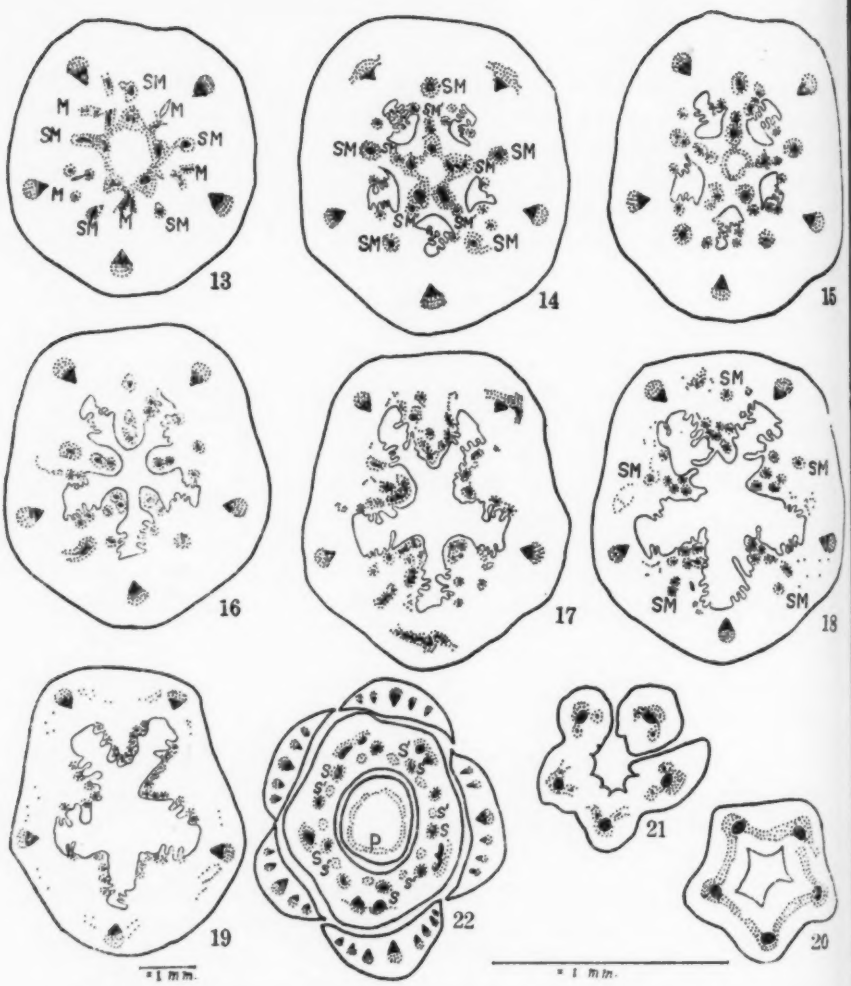
As the bundles SM proceed upward and outward, they give off a few small branches into the wall of the ovary and finally disappear completely beyond the ovule-bearing region (Fig. 19). It must be pointed out here that these bundles do not furnish any ovular traces. After their passing out, another set of five bundles SM′ separates off on the same radii (Figs. 14, 15). A major portion of the central stele is used up in the formation of these bundles. However, a small portion of procambial tissue always remains behind. All this converges to the centre and forms a plexus of residual tissue which disappears after traversing for a short distance (Fig. 15). Arber (1937) has also recorded such residual stelar tissue in a few species. As soon as they are formed the bundles SM′ start dividing. Generally they split into four bundles each, which in the beginning are arranged more or less equidistantly (Figs. 16, 17 and 18). But upward all of these migrate towards the ovule-bearing surface of the placentæ and divide into many branches (Figs. 17, 18). The ovules which are borne all over the inner surface excepting the midrib region receive their traces from these bundles. Some of the ovules which are near to the mid rib region are, however, receiving their traces from the remaining branches of the bundles marked as M.

It may be pointed out here that some of the bundles marked M (Fig. 13) in the region of their origin are often connected with bundles SM through small vascular bridges.

The five dorsal bundles together with some of their branches enter the stylar region (Fig. 20). All others have disappeared by this level. The dorsal bundles divide several times to supply the repeatedly dichotomised stigma lobes (Fig. 21).

The vascular supply of a staminate flower from a monœcious and a diœcious plant is almost the same. The pedicel here contains about 10 endarch bundles separated from each other by unequal parenchymatous strips. They divide in the cortex of the receptacle and give off five traces which divide sparingly before they enter the free parts of sepals. The remaining five traces are then set off on alternate radii. These divide more frequently and each has formed a more or less prominent arc of vascular bundles by the time the petal, in which it enters, separates off.

The remaining stelar tissue is again organised into a ring of ten bundles which are more or less equidistant from one another. A set of five staminal traces which diverges at a lower level supply the lower whorl of stamens



FIGS. 13–22. Diagrams of transverse sections of a gynœceum from base upward. Figs. 13–14. Note the marginal bundles dividing into two each, branches being separated by locule. Secondary marginal bundles of the second order (SM′) diverging on the same radii as SM. Fig. 15. Some amount of procambial tissue is left behind in the floral axis to disappear 'blindly'. Figs. 16–18. Show the bundles SM′ dividing and their migration towards the ovule-bearing surface. Note that the bundles SM have disappeared in Fig. 19 without supplying any ovule traces. Figs. 20–21. Sections through style and stigma region showing the five carpellary dorsals.

D—Dorsal bundle; M—Marginal bundle; SM—Secondary marginal strand; SM′—Secondary marginal strand of the second order; S—Staminal bundle; S′—Vascular stub; P—Vascular tissue for the pistillode.

while another higher up supplies the upper whorl of stamens. In flowers from a monœcious plant, in addition to ten staminal traces, there are ten vascular stubs which are procambial in nature and which disappear after continuing up for a short distance without entering into any organ. They are probably the stubs for the staminodes which no more exist, but which may have existed in the ancestral forms. After the separation of the staminal and staminodal traces some stelar tissue still remains in the centre. This is all procambial and enters the pistillode (Fig. 22).

In a hermaphrodite flower the vascular supply in the bract, bracteole, sepal and petal is almost similar to that in a female flower except for the fact that here the vascular bundles of petals, in the basal region, occur in a more or less complete ring because of partial gamopetaly.

The remaining stelar tissue resolves to form about 20 vascular bundles which arrange themselves in two rings of ten bundles each, those of the two rings occupying alternate radii. The bundles of the outer ring set off almost simultaneously to enter the stamens. The behaviour of the ten bundles of the inner ring is somewhat vague and irregular. Usually 3 or 4 bundles are first given off which may later on divide to form 5 carpellary dorsals. The placental vascular supply also does not follow any definite plan. Some of the bundles in the ring divide a number of times. The resulting branches enter the placental regions in varying numbers—four, three, or even fewer per placenta. The style, however, receives only five vascular bundles, the carpellary dorsals, and these, as usual, divide to supply the dichotomies of the stigmatic lobes.

Discussion

Sepals and Petals.—Five vascular bundles are given off from the stele each of which resolves to supply a sepal and a petal. Every sepal therefore receives only one trace as opposed to three in the related family Passifloraceæ (Puri, 1947). In view of the small size of the sepals in *Carica*, such a modification is easily understandable and it appears to me that it lends some support to the well-known assertion of Bower (1923) that in " any progressive evolution vascular structure follows, it does not dictate, external form." Puri (1951) while reviewing the literature on floral anatomy has also arrived at a more or less similar conclusion.

As has already been pointed out the corolla is partly gamopetalous in hermaphrodite and staminate flowers and polypetalous in pistillate flowers. Whether the fusion of petals is in any way connected with the production of stamens, I am not, as yet, in a position to say but the facts of the situation seem to point in that direction. It would be worthwhile to investigate this point further from the developmental point of view.

This feature may be significant from yet another point of view. Gamopetaly occurs occasionally in Passifloraceæ (Puri, 1947) and generally in Cucurbitaceæ (Judson, 1929). The occurrence of gamopetaly in staminate and hermaphrodite flowers of *Carica* does emphasize a closer affinity with the Cucurbitaceæ and Passifloraceæ. Further, if polypetaly and gamopetaly occur side by side in the same groups, as it does here, we have got to realize the limitations of our so-called taxonomic categories of Polypetaly and Gamopetaly. The researches of Douglas (1936) and Dickson (1936) on Primulaceæ also emphasize the same point.

Stamen.—All the ten staminal bundles arise at the same level but perhaps due to lack of space the two sets of 5 bundles each enter the stamens in two whorls. In the hermaphrodite flower, however, all the ten vascular traces enter the ten stamens simultaneously. This is perhaps connected with the comparatively large size of the flower here.

The occurrence of ten vascular stubs in a staminate flower from a monœcious plant seems to suggest that in ancestral forms the stamens might have been more than ten. The absence of such vascular stubs in staminate flowers from a diœcious plant may be of some significance in so far as it may indicate that in *Carica* diœcism is more specialized and reduced than monœcism. It may be pointed out here that staminodes have also been reported in certain species of the Cucurbitaceæ (Saunders, 1939) and Passifloraceæ (Puri, 1947).

Gynæceum.—The fact that some amount of vascular tissue does continue beyond the passing out of the last carpellary traces seems to indicate that carpels like any other floral structure are lateral organs. The pentacarpellary gynæceum has five large placentæ which extend all over the inner surface excepting a small area about the midrib. With the exception of the dorsal bundles and the bundles marked as SM, all others are involved in supplying ovular traces. As has already been pointed out there is little doubt that the bundles SM are secondary marginal strands. The description of bundles SM′ and M is, however, difficult and deserves some attention here.

The position and behaviour of the bundles marked M are rather significant. They arise just opposite the midrib bundles on the inner side of the locules. This is a position which is generally occupied by marginal bundles or placental strands in axile placentation (Puri, 1952 *a*). Soon after, these bundles split up into two each and pass out one on either side of a locule and furnish ovular traces in the basal region of the ovary. Near about the points of origin some of these bundles often appear as if connected with the secondary marginals SM. Such connections between bundles in the

placental region have also been observed in some species of the Cucurbitaceæ (Puri, 1952 *b*).

A little upward, however, bundles marked SM′ become differentiated and branch to supply ovular traces. These bundles alternate with the midrib bundles and occur on the inner side of the secondary marginal strands (SM). If bundles marked M are to be regarded as placental strands (composed of marginal bundles), bundles SM′ should be interpreted as secondary marginals of the second order, SM being of the first order. This appears to be an interesting situation for as has been shown by Puri (1952 *a*) it is uncommon, if not rare, for secondary marginals to be called upon to furnish ovular traces. May be that it is connected with the large size of the placentæ and with the large number of ovules they bear. Thus the vascular supply of the placental region is of double origin, partly from the marginal bundles and partly from the secondary marginal strands of the second order (SM′). A more or less similar situation has been observed in some species of the Cucurbitaceæ (Puri, 1952 *b*).

Placentation.—The placentation in *Carica papaya* seems to be superfical, the ovules occurring all over the inner surface except a small region about the midrib. If vascular bundles are worth anything it is tempting to speculate a little at this juncture into the past history of placentation in the Caricaceæ. The origin of the placental bundles (M) opposite to the dorsal bundles of carpels seems to indicate that the ancestors of *Carica papaya* might have had axile placentation. This changed over to parietal placentation as the placental strands split apart and finally the parietal placentæ became so extended that they covered major portion of internal surface of the carpellary wall. As they extended side ways the marginal bundles alone became insufficient for supplying ovuler traces. The secondary marginal strands were then called upon, as it were, to furnish additional ovular traces. Thus it appears that superficial placentation in *Carica* is derived from axile through parietal placentation. This is in conformity with the conclusions already arrived at by Puri (1952 *a*) regarding the evolutionary trends in placentation.

Any discussion of the affinities of the family Caricaceæ will be premature at this stage. It is advisable to postpone it until some more material has been studied. But it can perhaps be pointed out that the structure of the flower in general and that of the gynæceum in particular supports a position for the Caricaceæ near the Passifloraceæ and Cucurbitaceæ. A more or less similar inference has been arrived by Foster (1943) and others.

SUMMARY

1. External morphology and vascular anatomy of the flowers of *Carica papaya* have been described and the results analysed.

2. The general occurrence of partial gamopetaly in staminate and hermaphrodite flowers of *Carica* has been compared with the similar condition in the Cucurbitaceæ and Passifloraceæ.

3. Some morphological and anatomical differences in the staminate flowers from diœcious and monœcious plants have been noted and it has been tentatively suggested that in *Carica* diœcism is more specialized and reduced than monœcism.

4. The vascular supply of sepals is believed to have undergone reduction corresponding to external form.

5. The vascular supply of the placenta is interesting in so far as it is furnished partly by marginal bundles and partly by secondary marginal strands. This has been made the basis for the suggestion that superficial placentation in *Carica* has been derived from axile placentation.

6. The comparative morphology and vascular anatomy, specially of gynæceum of *C. papaya* seems to justify the position of the Caricaceæ near the Passifloraceæ and Cucurbitaceæ.

ACKNOWLEDGEMENTS

I am sincerely thankful to my venerable teacher Dr. V. Puri, D.SC., F.A.SC., F.B.S., for the valuable guidance and encouragement he gave me during the course of this study and for permission to carry on this work when I was holding an appointment of Research Assistant with him. I also express my thanks to Mr. D. N. Sharma, M.SC., for the help he rendered in collecting material.

LITERATURE CITED

1. Arber, A. .. "Floral anatomy and its morphological interpretation," *New. Phyt.*, 1933, **32**, 231–41.
2. ———— .. "The interpretation of the flower. A study of some aspects of morphological thought," *Bio. Rev.*, 1937, **12**, 157–84.
3. Bower, F. O. .. *The Ferns (Filicales)*, 1923, **1**, Cambridge.
4. Dickson, J. .. "Studies in Floral Anatomy. III. An interpretation of the gynæceum in the Primulaceæ," *Amer. Jour. Bot.*, 1936, **23**, 385–93.
5. Douglas, G. E. .. "Studies in the vascular anatomy of the Primulaceæ," *Ibid.*, 1936, **23**, 199–211.
6. Engler, A. and Prantl, K. .. *Die natürlichen Pflanzenfamilien.* 1925, Band 21, Leipzig.
7. Foster, L. T. .. "Morphological and cytological studies on *Carica papaya*," *Bot. Gaz.*, 1943, **105**, 116–26.

8. Gundersen, A. .. *Families of Dicotyledons*, 1950, Chro. Bot. Co., U.S.A.
9. Hooker, J. D. .. *The Flora of British India*, 1879, **2**, London.
10. Hutchinson, J. .. *The Families of Flowering Plants. Dicotyledons*, London, 1926, **1.**
11. Judson, J. E. .. "Morphology and vascular anatomy of the cucumber," *Amer. Jour. Bot.*, 1929, **16**, 69–86.
12. Puri, V. .. "Studies in the floral anatomy. IV. Vascular anatomy of the flower of Certain Species of Passifloraceæ," *Ibid.*, 1947, **34**, 562–73.
13. ———— .. "The role of floral anatomy in the solution of morphological problems," *Bot. Rev.*, 1951, **17**, 471–553.
14. ———— .. "Placentation in Angiosperms", *Ibid.* (in press), 1952 *a*.
15. ———— .. "Studies in floral anatomy. VII. On placentation in certain species of the Cucurbitaceæ" (in press), 1952 *b*.
16. Rendle, A. B. .. "Classification of flowering plants," *Dicotyledons*, 1925, **2**, Cambridge.
17. Saunders, E. R. .. "The neglect of anatomical evidence in the current solutions of problems in systematic botany," *New Phyt.*, 1939, **38**, 203–09.
18. Van Tieghem, Ph. .. *Elements de Botanique. speciale* Band II, 1918, Paris.
19. Warming, E. and Potter, M. C. .. *A Hand-Book of Systematic Botany*, 1932, London.
20. Wettstein, R. .. *Handbuch der systematischen Botanik*, 1935, Band II, Leipzig.
21. Worsdel, W. C. .. *The Principles of Plant Teratology*, 1916, **2**, London.

THE AMYLOSE AND THE AMYLOPECTIN CONTENTS OF RICE AND THEIR INFLUENCE ON THE COOKING QUALITY OF THE CEREAL

By B. Sanjiva Rao, F.A.Sc., A. R. Vasudeva Murthy and R. S. Subrahmanya

(Indian Institute of Science, Bangalore)

Received February 23, 1951

Introduction

Hundreds of varieties of rice are known to exist. These varieties exhibit on cooking marked differences in quality. Attempts to correlate quality of a rice with its chemical composition have not so far been successful. According to B. S. Rao,[1] the differences in quality are mainly to be attributed to differences in colloidal structure. Rao has shown, that the extent of swelling of any variety of rice on cooking, is an index of its quality. He has developed a convenient and accurate method of estimating the swelling. This swelling is quantitatively expressed as the " Swelling Number " (S.N.) of a rice. The swelling number, is the weight of water imbibed by 100 grams of rice when cooked in water at 98° C. under standard conditions. It has been found that it is possible to alter the swelling number of rice by processing, *e.g.*, by par-boiling.

The starch of rice, like all other starches, consists of amylose and amylopectin. W. N. Haworth, *et al.*,[2] have confirmed that amylose is composed largely of unbranched chains of 100 or more glucose members, while amylopectin has a laminated or branched structure, composed of unit chains of about 20 glucose residues. Amylose produces a blue colour, while amylopectin yields reddish coloration, with iodine.

The present investigation was undertaken to find out if there was any relationship between the swelling number of a rice and its amylose and amylopectin contents. It was found that there were varietal differences in the amylose and the amylopectin contents of rice. Whether processing of rice (which brings about a change in the swelling) would also affect the amylose-amylopectin ratio, was determined.

Experimental

(A) *Fractionation of Amylose and Amylopectin from Rice*

Pure amylose and amylopectin samples were required for the standardisation of the method of analysis. These fractions were obtained from rice.

The more important methods that have been employed, from time to time, for the fractionation of starch are the following:—

(1) *Aqueous leaching.*[3, 4, 5]—This method depends on the extraction of the swollen granules, with successive portions of water, until all the soluble portion is removed. The insoluble portion is separated by sedimentation, filtration or centrifugation. This method is tedious, sterile conditions are difficult to maintain, and the separation is imperfect.

(2) *Electrophoresis.*[6, 7]—This method depends upon the polarity imparted to one or the other of the fractions, by the presence of non-carbohydrate substances. The separation is slow and incomplete. Furthermore, there is a considerable amount of degradation of the starch.

(3) *Selective retrogradation.*[8, 9]—When a starch sol is allowed to stand, the amylose fraction slowly retrogrades and precipitates from solution. This method is usually employed on starches solubilized by hydrolytic action. Fractionation is superficial, since the amylopectin fraction acts as a protective colloid. Moreover, considerable co-precipitation occurs.

(4) *Selective adsorption.*[10, 11, 12]—Some adsorbents like cotton, cellulose pulp, charcoal, activated alumina, bentonite and fuller's earth, preferentially adsorb the amylose fraction which can be eluted with hot water. Although this method involves no chemical degradation and yields relatively pure amylopectin fraction, the amylose fraction is badly contaminated with amylopectin.

(5) *Enzymic method.*[13, 14]—The amylose fraction can be removed through hydrolysis with various enzymes. But it is now recognised that amylopectin is also affected to an extent of about 50%. Consequently this method is also unsuitable.

(6) *Selective precipitation.*[15]—The only suitable method seems to be selective precipitation by polar organic substances. This method, which has been developed by Schoch, gives without any chemical degradation, both fractions of maximum purity. In the present investigation, the method of Schoch[16] has been employed.

The fractionation involves dispersion of starch by autoclaving, precipitation of the amylose by the addition of butyl alcohol, separation of the precipitate by centrifuging and the final purification of amylose by " Recrystallisation ".

A mixture of amyl alcohol and butyl alcohol is found to give better yields than butyl alcohol and is therefore preferred. During autoclaving, the fat gets hydrolysed and develops acid, which aids glucosidic hydrolysis

of starch. Consequently, defatted starch is preferred in all the physico-chemical investigations. Schoch recommends methyl alcohol as the best defatting agent for the cereal starches.

100 g. of powdered rice (fully polished) was extracted in a Soxhlet with methyl alcohol for 24 hours. The defatted starch granules were dried in air and finely powdered to pass through 200 mesh.

20 g. of the defatted rice flour was added to 75 ml. of water at 70° C. and kept at this temperature for 10 minutes. This suspension was slowly added with constant mechanical stirring to a boiling mixture of one litre of water and 100 ml. of butyl alcohol, heated on the steam-bath. The paste was then autoclaved for 3 hours at 18–20 lb. pressure (124–25°).

The hot starch sol was passed through the Sharples continuous-flow supercentrifuge to remove traces of cellular material and other impurities. To the clear sol was then added 100 ml. of a hot, 1 : 1 mixture, of butyl and amyl alcohols. The solution was allowed to cool slowly to room temperature (without agitation), over a period of 36 hours. To effect slow cooling, the beaker was kept wrapped in flannel.

When the temperature was about 50° C., the precipitated fraction separated out as a gelatinous floc. The mixture was then stirred at room temperature for about 3 hours to break up the mass and refrigerated overnight. The starch mixture was then centrifuged at about 3,000 r.p.m. in a centrifuge. The supernatant liquid was decanted carefully. The precipitated material was again suspended in cold water (previously saturated with butyl alcohol) and then centrifuged. The washing was repeated, until the supernatant liquid indicated no turbidity with methyl alcohol, added in excess.

The crude fraction was further purified by repeated " Recrystallisation " from boiling water in the presence of excess of butyl alcohol. The purified amylose fraction obtained in this way, was preserved under methyl alcohol.

The amylopectin fraction could readily be removed from the centrifuge by flocculating it with excess of methyl alcohol. The wet precipitate was triturated with fresh portions of methyl alcohol, to effect its dehydration. The purified amylopectin fraction obtained in this way was also preserved under methyl alcohol.

(B) *Analytical Procedure*

Amylose is stained blue by iodine while amylopectin is stained purple to red. F. L. Bates[17] and others attributed this striking difference in behaviour to a difference in the ability of amylose and amylopectin to bind

iodine in complex formation. So they titrated solutions of amylose and amylopectin with iodine potentiometrically. Their results indicate that the "activity" of iodine in an amylose solution remains constant upon addition of iodine until complex formation is complete, while there is a continual rise in the iodine activity, when iodine is added to an amylopectin solution. Starches containing both components show an inflexion in the curve: potential *vs.* Iodine titre. This inflexion point is a measure of the amylose fraction present in starch.

In the method developed by Bates and co-workers,[17] the starch sample is dissolved in dilute potassium hydroxide solution, neutralised with hydriodic acid and titrated potentiometrically with a solution of iodine in potassium iodide. Since hydriodic acid requires frequent redistillation over red phosphorus, Schock, *et al.*,[18] have modified the process by employing hydrochloric acid to neutralise the alkaline starch solution. The titration is then carried out with a solution containing iodine, potassium iodide and potassium chloride. They point out that completely defatted cereal starches have to be used, in order to avoid interference due to the adsorption of fatty acids on starch. In the present investigation, Schoch's method, in its modified form, was employed.

Very dilute solutions—0·01 to 0·04%—have to be employed as it is essential that starch should be in a completely dispersed state. As the amount of iodine taken up by starch is influenced by the iodide concentration, it is necessary to keep the iodide concentration constant, to obtain concordant results.

0·01 to 0·04 g. of starch was dispersed in 10 ml. of 0·5 N potassium hydroxide and slightly warmed to get an effective dispersion. The solution was diluted with distilled water, and made neutral to methyl orange by the addition of 0·5 N hydrochloric acid. 10 ml. of 0·5 N potassium iodide was then added and the solution made up to 100 ml. with distilled water.

The solution thus prepared, was slightly acidic and 0·05 N with respect to potassium chloride and potassium iodide. This solution was titrated potentiometrically with an iodine reagent 0·001 N to iodine, 0·05 N to potassium iodide and 0·05 N to potassium chloride. The potentiometric titrations were carried out with a vernier type potentiometer which could read to a tenth of a milli-volt.

Using the above procedure, different varieties of rice were analysed. The results are given in Table I. Cured, Calcured and Parboiled forms from the same sample of "Madivala Paddy" were also analysed to find out whether the different methods of processing the cereal, affect the amylose and amylopectin contents in rice.

Calcured Rice.[19]—Paddy was soaked in a 0·2 molar solution of calcium chloride (pH adjusted to 4·5) at 65° C. for 2 hours. The paddy was removed from this hot solution and steamed for 10 minutes to partially gelatinize the starch. The paddy was then dried.

Cured Rice.—This was obtained in the same way as indicated above for calcured rice, but water was used for soaking the paddy.

Parboiled Rice.—Parboiled rice was prepared by soaking the paddy in cold water for 24 hours and boiling the paddy with water for half an hour. The paddy was then dried.

All the samples of paddy were husked and the rice fully polished. The samples of rice were defatted by extraction with methyl alcohol in a Soxhlet for 24 hours.

Standardisation of the Analytical Procedure

Fig. 1 shows the changes in potential when 0·001 iodine is added to solutions of amylose and amylopectin in 0·05 N potassium iodide solution.

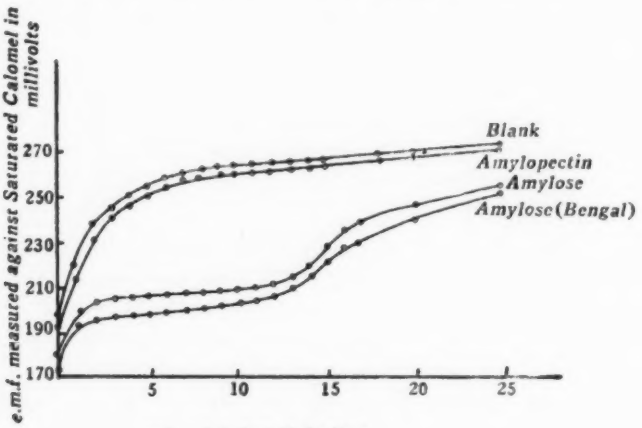


FIG. 1

The curve for the potassium iodide solution, no starch being present, indicates the concentration of free iodine, corresponding to any potential. The curve for amylopectin shows that all the iodine adsorbed is bound by amylopection. For amylose, there is an inflection point and at this stage, all the iodine present, is in the form of a complex with amylose. On further addition, the iodine is merely adsorbed, as can be deduced from the proportional increase in the concentration of free iodine. The value of bound iodine is obtained by subtracting from the total iodine, the free iodine corresponding

to any potential. In Fig. 2 the number of grams of iodine bound per gram of amylose have been plotted against the concentration of iodine. Estimated

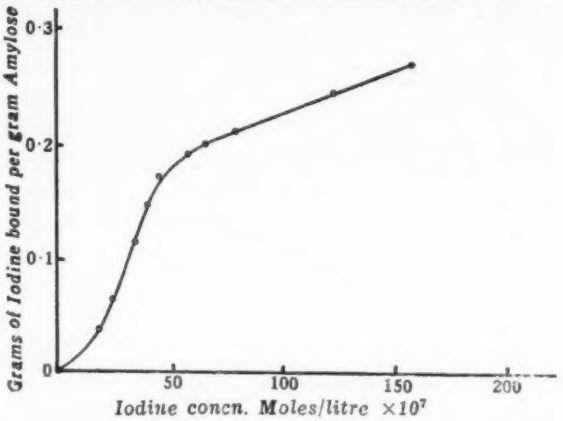


FIG. 2

in this way, amylose from rice is found to take up 18·2% of iodine. An amylose sample, supplied by Bengal Immunity Research Institute, has been found to take up 18·4% of iodine.

By determining the inflection point obtained with a mixture of amylose and amylopectin, the amylose content of the sample can be computed. The values of amylose in various samples of rice are given in Table I. The titration curves obtained with a few typical samples of rice are indicated in Fig. 3.

TABLE I. *Effect of Time of Cooking on the Swelling of Different Samples of Rice*

Sl. No.	Duration of cooking (in minutes)	Swelling number of "Madivala Sanna"	Swelling number of "C. Sanna"*	Swelling number of "D.S. Medium"†	Swelling number of "D.S. Coarse"‡
1	20	255	240	225	200
2	30	305	285	240	225
3	45	349	323	282	250
4	60	396	362	320	276
5	90	452	420	365	305

* "C. Sanna"—Coimbatore Sanna; ‡"D.S. Medium"—Dabbalsala medium; †"D.S. Coarse"—Dabbalsala coarse.

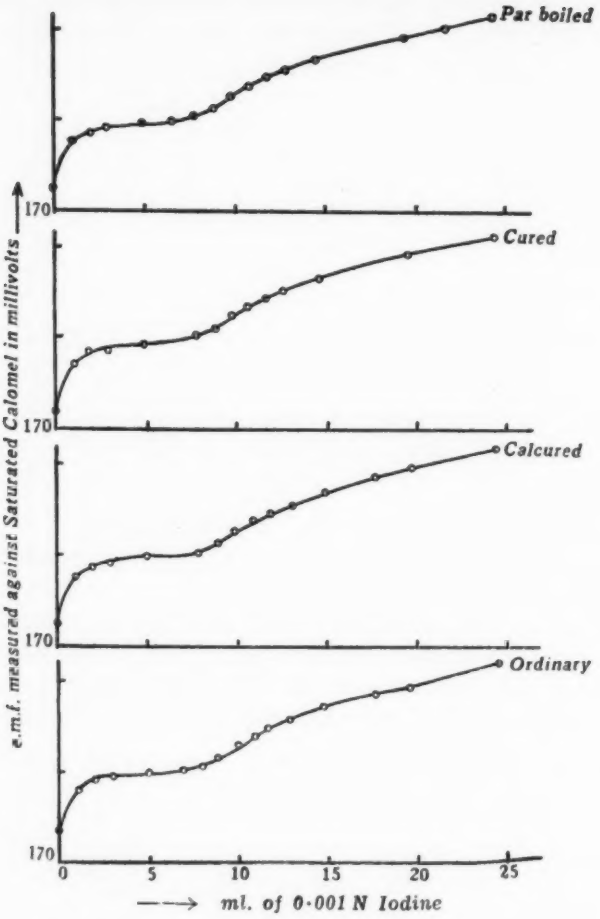


FIG. 3

(C) *Swelling Numbers*

The "Swelling Numbers" (S.N.) of different varieties of rice were determined by the procedure described by B. S. Rao.[1] The apparatus consisted of a three-necked flask through one neck of which was attached a water-cooled condenser and through the other a pyrex test-tube containing 10 ml. of distilled water. A thermometer passing through a rubber-stopper was fitted into the third neck. The test-tube was loosely plugged into the neck with cotton wool. When the water in the test-tube just began to boil, a known weight (1 to 1·3 g.) of the fully polished rice sample was intro-

duced through a wide-mouthed funnel. The funnel was removed and the cotton plug was inserted.

Exactly, 30 minutes later, the test-tube was withdrawn from the steam-bath and filled with ice-cold water. The water was drained off by transferring the contents of the test-tube into a perforated thimble of porcelain. The thimble was then kept for about five minutes in ice-cold distilled water. It was withdrawn, and after the water had drained off, the rice was spread over moist chamois leather kept on a porcelain plate and the excess of moisture was removed by placing over the rice another piece of moist chamois leather and applying gentle pressure. Moist chamois leather was employed, as it was found to be the most convenient way of removing surplus water. The swelling numbers could be determined with an accuracy of 1%.

The effect of time of cooking on the swelling of rice, was studied by cooking a sample for different periods. The swelling number could not be determined beyond 90 minutes as there was disintegration in the rice grain. Table I indicates the effect of time of cooking on the swelling numbers of different samples of rice.

TABLE II. *Moisture, Amylose Content and Swelling Number of Various Samples of Rice*

Sl. No.	Rice sample	Moisutre %	Swelling number	% of Amylose
1	"Madivala Sanna"	14·8	305	17·5
2	"Mysore Kaddi"	15·0	304	17·5
3	"B. 1399 Putta Sanna" ..	15·0	292	15·0
4	"Chikka Dabbalasala" ..	15·3	242	7·0
5	"Makka Dabbalasale" ..	15·0	272	12·0
6	"Tolaga"	15·6	266	10.5
7	"C. Sanna"	15·1	285	14·5
8	"Siddasale"	15·4	260	10·0
9	"Kumbri Halaga"	15·0	260	10·0
10	"Neerudabbalasale"	15·0	258	10·0
11	"S. 661"	15·4	275	12·5
12	"C.S. Fine"	15·3	305	17·5
13	"S. 749"	15·4	292	15·0
14	"Dabbalasala (Medium)" ..	14·7	240	7·0
15	"Dodda Hegge"	15·5	282	14·0
16	"Rathachoodi"	15·2	300	17·0
17	"Dabbalasala (Coarse)" ..	15·0	225	4·5
18	"B. 281 Koppa"	15·4	265	10·5
19	"Makki D.S. Paddy"	15·6	286	14·5
20	"Bilikan Hegge"	15·0	260	10·0
21	"Varedabbalasale"	15·2	256	9·5
22	"Dodda Dabbalasala" ..	15·2	200	0·0

(D) *Estimation of Moisture*

Moisture was estimated by employing the Carter-Simon rapid moisture tester. The oven was kept at 155° C. Drying time was 30 minutes. The moisture content was calculated on the basis of weight of " dry " rice, present in paddy, taking into account the weight of husk.

In Table II are given the moisture and amylose contents and the swelling numbers of various samples.

It is interesting to note that the coarsest variety of rice investigated, has no amylose at all. This rice is similar to the waxy rice reported by Schoch.[16] Superior varieties are richer in amylose. No marked difference is noticed in the moisture content of different samples of paddy under identical conditions of humidity.

DISCUSSION

The experimental results recorded in this paper, indicate that there is a close correlation between the " Swelling Number " of raw polished rice and its amylose content. The higher the percentage of amylose in a rice, the greater is the swelling. According to modern views, amylose is a linear chain polymer, while amylopectin is a branched chain polymer. During its growth in the rice plant, the rice grain seems to acquire its amylopectin by the action of certain enzymes on amylose. Haworth[2] has discussed the relationship between amylose and amylopectin. It is of interest to note that the amylose content of rice does not alter when it is cured.

Rice having a high swelling number is liked by consumers as such rice is soft when cooked. The swelling number may therefore be considered to be an index of the quality of rice. The determination of swelling numbers is easily carried out and highly reproducible values are obtained. The correlation between swelling number and the amylose content of rice is of significance. It may be pointed out that the swelling number is an index of the rigidity of structure of the rice grain. If the grain has comparatively a loose structure, the swelling number is high. Amylose, being a linear chain polymer, makes the structure of the rice grain less rigid and this accounts for the higher swelling number of varieties having higher amylose content. The looseness of structure caused by amylose may however be modified by the curing of rice, as in parboiling, where the paddy, on soaking in water, is subjected to " wet heat ", *i.e.*, the soaked paddy is boiled or steamed for 15 to 20 minutes.

B. S. Rao has emphasised the fact that rice is to be looked upon as a colloidal system analogus to the hydrogel of silicic acid. It is well known that when the hydrogel of silicic acid is subjected to " wet heat " that is,

heated under conditions in which there is no loss of moisture, the hydrogel acquires rigidity of structure and becomes a Xerogel. In fact, this is the technical method for the production of silica gel on the large scale. Similarly, when the hydrogel of starch (containing both amylopectin and amylose) is subjected to wet heat, we get a product having greater rigidity. The rigidity of structure in the rice grain can, in fact, be controlled. For instance, in the case of rice parboiled by the indigenous process, the swelling number drops to 265 (Table III) while rice that has been subjected to wet heat under controlled conditions, can be made to yield a product having practically the same swelling number as raw rice.

TABLE III. *Swelling Number and Amylose Content of Processed Rice from "Madivala Sanna"*

Sl. No.	Rice sample	Swelling number	Amylose content
1	"Raw polished"	305	17·5
2	"Cured with water"	304	17·5
3	"Calcured"	305	17·5
4	"Parboiled" (Indigenous method)	265	17·5

The fact that the rigidity of the rice grain can be controlled by wet heat treatment is of considerable practical importance as it enables one to get rice, in which vitamins are fixed in the endosperm, and which has the desired softness in cooking. It may be pointed out that rice cured by the indigenous "parboiling" process is generally "overcured". This partly accounts for the unpopularity of parboiled rice in certain parts of the world.

SUMMARY

1. Amylose and amylopectin fractions have been isolated from rice by a slight modification of the butanol fractionation method of Schoch. This method has been found to give good yields.

2. The amylose and the amylopectin ratios of the different varieties of rice have been estimated by the potentiometric titration of starch solutions with iodine.

3. The cooking quality of the different varieties of rice has been determined by employing the "Swelling Number" method of B. S. Rao.

4. It has been found that with an increase in the amylose content, the swelling number of rice increases.

5. No change in the amylose content takes place, when rice is parboiled though there is a marked fall in the Swelling Number. Other ways of curing rice do not also affect the amylose content.

ACKNOWLEDGEMENT

The authors thank the Director of Agriculture in Mysore, for the various samples of rice and to the authorities of Bengal Immunity Research Institute, for a sample of amylose. The major part of the investigation described in this paper was conducted at the Central College, Mysore University, Bangalore.

REFERENCES

1. B. Sanjiva Rao .. *X Congresso Internazionale Di Chimica*, Rome, 1938, **4**, 550-58.
2. W. N. Haworth .. *J. Chem. Soc.*, 1946, 543.
3. Z. Gatin-Gruzewska .. *Compt. Rend.*, 1908, **146**, 540-42.
4. M. E. Baldwin .. *J. Am. Chem. Soc.*, 1930, **52**, 2907-19.
5. K. H. Meyer, W. Brentano and P. Bernfeld .. *Helv. Chim. Acta.*, 1940, **23**, 845-53.
6. M Samec and H. Hærdtl .. *Kolloidchem. Beihefte*, 1920, **12**, 281-300.
7. T. C. Taylor and H. A. Iddles *Ind. Eng. Chem.*, 1926, **18**, 713-17.
8. L. Maquenne .. *Compt. Rend.*, 1903, **137**, 88-90, 797-99.
9. Z. Gatin-Gruzewska .. *Ibid.*, 1911, **152**, 785-88.
10. C. Tanrel .. *Ibid.*, 1914, **158**, 1353-56.
11. E. Pacsu .. *J. Am. Chem. Soc.*, 1941, **63**, 1168.
12. T. J. Schoch and C. B. Williams .. *Ibid.*, 1944, **66**, 1232.
13. A R. Ling and D. R. Nanji .. *J. Chem. Soc.* 1923, **123**, 2666-88.
14. S. B. Schryver and E. M. Thomas .. *Biochem. J.*, 1923, **17**, 497-500.
15. T. J. Schoch .. *Cereal Chem.*, 1941, **18**, 121.
16. ———— .. *J. Am. Chem. Soc.*, 1942, **64**, 2957-61.
17. F. L. Bates, D. French and R. E. Rundle .. *Ibid.*, 1943, **65**, 142-48.
18. E. J. Wilson, Jr., T. J. Schoch and C. S. Hudson .. *Ibid.*, 1943, **65**, 1380-83.
19. B. Sanjiva Rao .. *Proceedings of the 35th Indian Science Congress* (Patna), Presidential Address, Part II, 1948, 259-70.

FERTILIZATION VALUE OF TANK SILTS

II. Phosphorus and Reproductive Vigour of *Oryza sativa* L.

By C. S. Venkata Ram
(*University Botany Laboratory, Madras*)
AND
K. V. Srinivasa Pai
(*Madras Christian College, Tambaram*)

Received April 17, 1952
(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

In an earlier communication (Venkata Ram and Pai, 1951) the presence of N and K in tank silts in catalyzing growth in the rice plant was reported. The present investigation was undertaken to determine the role of phosphorus in relation to ammoniacal and nitrate nitrogen, organic matter, iron and manganese in the silts in influencing the reproductive vigour of the rice plant and the results are discussed below.

Materials and Methods

In addition to silt samples A and B used earlier by the authors, another sample C from Triplicane temple tank, Madras City, was also included in this investigation and the experimental procedure largely remained the same (*loc. cit.*) with the exception that tin cans of $9'' \times 6'' \times 6''$ size quoted with inert bitumen paint were used as containers for the silt soil admixture. At first 50 seeds were sown in each can, maintaining three replicates for each series, and later thinned out to 20 seedlings per can, the entire experiment being run in the open under field conditions. Chemical assays of the silt samples were carried out by following standard methods and the results are presented in Table I. The vigour of rice plants 90 days after sowing in soils containing 15 per cent. silt each of the three samples are presented in Plate III and Text-Fig. 1. The control plants were not photographed since they behaved similarly to those grown in silt sample C.

Discussion of Results

Growth stimulated by silt application at either 10 or 15 per cent. of sample C was practically negligible when compared to the control (garden soil); the other two samples A and B markedly improved growth as is seen in Plate III. The chemical composition of the three silts manifested striking

TABLE I

Chemical composition of the silt and soil samples determined quantitatively

	Presence of various constituents in the samples represented as milligram per cent.			
	Garden soil	Silt samples		
		A	B	C
Total soluble salts ..	280	452	312	268
Chlorides	21	20	18	28
Total Nitrogen	112·8	362·4	240·6	185·4
Organic matter	0·64*	2·54*	3·24*	1·34*
Ammonium Nitrogen ..	86·5	155·6	186·6	150·8
Nitrate Nitrogen ..	4·6	22·4	14·6	12·6
Potassium (K_2O) ..	228	432	328	208
Phosphorus (P_2O_5) . ..	64	166	147	108
Iron (Fe_2O_3)	760	540	430	260
Manganese	12·68	3·4	3·84	1·16

* Gram per cent.

differences. Both samples A and B were found to contain more total nitrogen, potassium, phosphorus and organic matter than sample C. Sample C, however, indicated higher figures for total nitrogen, ammonium and nitrate nitrogen, phosphorus and organic matter than unmanured garden soil although presenting lower figures for K, Fe and Mn.

Further interest centred round the fact that sample C, although containing considerable quantities of N, P, Fe and Mn, yet had very low K. Indeed, the failure of sample C to catalyze growth of the rice plants to any appreciable extent could be attributed to lack of potassium only (being even lower than in garden soil) since this silt was almost as rich as sample A in

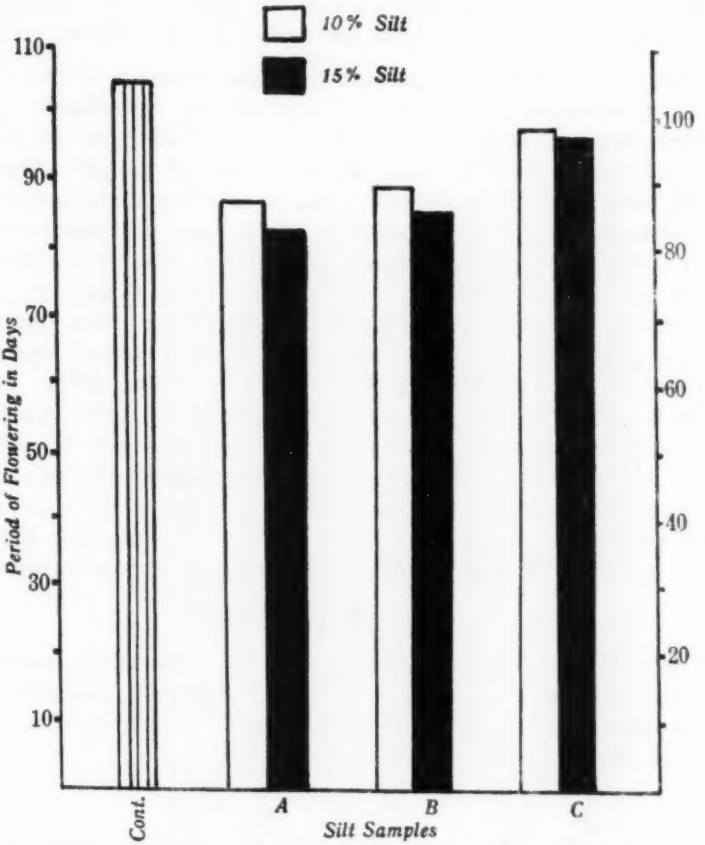


FIG. 1 shows earliness of flowering of rice plants grown in silt samples A, B and C over those grown in unamended control.

ammoniacal nitrogen which is known to be essential to rice plants during initial growth (Dastur and Malkani, 1933). It should, therefore, be possible within reason, to make sample C a complete fertilizer by enriching it with optimum amounts of K, and only further work will show the practicable application of this hypothesis.

Regarding Fe and Mn contents of the silts, the lack of these elements rather than their abundance appears to have ameliorated growth of the plants because the substrate to which these were supplied was enriched of these elements. It is recognized that the action of Mn in the plant is linked with that of nitrogen, whereas that of Fe is linked with K (Russel, 1937). Thus

plentiful Fe and Mn in the garden soil and N and K in the silts might have made silt *plus* garden soil an ideal and well balanced nutritive substratum.

Samples A and B were complete fertilizers inasmuch as they not only stimulated vegetative growth but also catalyzed the reproductive vigour of the plants to a considerable extent. Earliness in flowering of the rice plants grown in either 10 or 15 per cent. silt amendment of samples A and B was marked, being 15 to 20 days ahead of that in the control, the earliness being three weeks in the case of sample A at 15 per cent. silt amendment (Text-Fig. 1). The third sample C did not contribute in any appreciable measure to early flowering. Undoubtedly, the presence of large amounts of phosphorus in the silt samples played a part in early flowering, this element being known to govern the reproductive phase of the plant life-cycle and also the uptake of nitrogen by the rice plant (Sircar and Sen, 1941). However, interest has been roused by recent reports (Hamence, 1945; Chaplin and Regan, 1945) that sludges, sewage and silts contain growth hormones and it may be that the silts employed in the present investigation also contained these hormones. Nonetheless, the striking earliness in flowering achieved by the application of these silts in conjunction with their rich contents of organic matter, N, K and P indicates further investigations in this field.

ACKNOWLEDGEMENTS

The authors are deeply indebted to Prof. T. S. Sadasivan and Dr. S. V. Anantakrishnan, F.A.SC., for their kind help in making available facilities and for their interest in this work. We thank the Government of India for award of scholarships.

REFERENCES

Chaplin, C. A. and Regan, C. J. .. "Investigations of plant-growth substances (auxins) in sewage products," *J. Soc. Chem. Ind. Lond.*, 1945, **64**, 331–32.

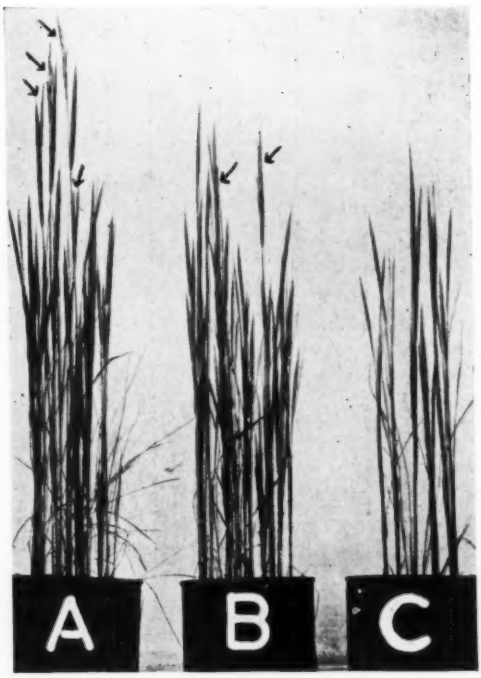
Dastur, R. H. and Malkani, T. J. .. "The intake of nitrogen by the rice plant (*Oryza sativa* L.)," *Ind. J. agric. Sci.*, 1933, **3**, 157–206.

Hamence, J. H. .. "The occurrence of auxins in organic manures," *J. Soc. Chem. Ind. Lond.*, 1945, **64**, 147–48.

Russel, E. J. .. *Soil Conditions and Plant Growth*, Longmans, Green & Co., Lond., 1937.

Sircar, S. M. and Sen, N. K. .. "Studies in the physiology of rice, I. Effect of phosphorus deficiency on growth and nitrogen metabolism in rice leaves," *Ind. J. agric. Sci.*, 1941, **11**, 193–204.

Venkata Ram, C. S., and Pai, Srinivasa, K. V. .. "Fertilization value of tank silts," *Proc. Indian Acad. Sci.*, 1951, **34 B**, 109–13.



Showing 90-day old paddy seedlings in silt samples A, B and C. Note: Arrows indicate ear heads.

ADDITIONS TO THE FUNGI OF MADRAS—XIII

By T. S. Ramakrishnan, F.A.Sc., K. V. Srinivasan and N. V. Sundaram

Received March 11, 1952

Sclerospora graminicola (Sacc.) Schr.

Butler, E. J. .. *Mem. Dept. Agric. India, Bot. Ser.*, 1907, **2**, 1, 24.

On *Panicum miliaceum* L. (Gramineæ) causing downy mildew, near Coimbatore, 24-5-51, T. S. Ramakrishnan.

Downy white growth of the conidial state was first noticed on both surfaces of the leaves and in the course of a fortnight the leaves turned yellow and then brown and the plants dried up in patches even before flowering. In some plants the symptoms were exhibited as yellowish long stripes. Ears were produced in some plants but the spikelets were chaffy and there was no grain setting. In most cases, whole plants were involved. In a few, partial infection of the plants was prevalent the rest of the plant forming normal ears. Shredding of the leaf was evident only rarely. Oospores were found in large numbers in the tissues of the leaves which had turned brown or become shredded.

The fungus closely resembles the one noticed on *Pennisetum typhoides* Stapf. and Hubb. in the measurements of the sexual and asexual reproductive bodies. The rapidity with which the disease spread over the whole field and the partial infection of several plants indicated that besides primary infection secondary spread through the agency of the sporangia should have taken place.

Pseudoperonospora cubensis (Berk. and Curt.) Rost.

Butler, E. J. .. *Fungi and disease in plants*, 1918, 311.

On leaves of *Luffa acutangula* Roxb. (Cucurbitaceæ), Coimbatore, 25-12-51, T. S. Ramakrishnan.

This fungus causes the downy mildew of several cucurbitaceous hosts. In Siddout (Cuddapah District) melon (*Cucumis melo* L.) is often affected by this fungus. At Coimbatore, the same fungus was recorded on *Cucumis sativus* L., *Trichosanthes anguina* L. and *Cucumis melo*. In order to find out

whether any specialisation was prevalent in this species, cross inoculation experiments were carried out with the fungus on the different hosts. The fungus readily passed on from one to the other hosts and *vice versa*. But *Cucurbita maxima* Duch. which was growing alongside them was quite free from infection in nature. Even when artificially inoculated with the fungus from the other hosts no instance of positive infection was evident. The plant has however been recorded as a host for this fungus from other parts of India. The strains growing on *Cucumis* and other hosts recorded above did not pass on to *Cucurbita maxima*, showing thereby the existence of specialisation in this pathogen. According to Fitzpatrick this fungus is included in the genus *Peronoplasmopara* as the name *Pseudoperonospora* by Rostowzew did not satisfy the requirements of Article 38 of International Rules of Botanical Nomenclature.

Claviceps paspali Stev. and Hall.

Sprague, R. .. *Diseases of cereals and grasses.* The Ronald Press Co., New York, 1950, 60.

On the inflorescence of *Paspalum dilatatum* Poir. (Gramineæ), Kodaikanal, 21–12–51, N. V. Sundaram.

Paspalum dilatatum is the South American fodder grass. Attempts have been made to introduce this grass on the hill stations in South India and it is common in and around Ootacamund and Kodaikanal and has occasionally run wild. In December 1951, this grass was found to be heavily infected at Kodaikanal. The honeydew stage was the most prevalent state. Cross inoculations with this fungus on the ears of *P. scrobiculatum* L. were highly successful. Both the conidia and sclerotia were formed on this host. On analyses the sclerotia were found to contain the alkaloid ergotoxine. Stock poisoning has been reported from other countries caused by grazing on infected ears of *P. dilatatum*. The spread of *P. dilatatum* as a fodder grass in localities liable to ergot infection is to be watched for freedom from ergot.

Epichloe typhina (Fr.) Tul.

Sprague, R. .. *Diseases of cereals and grasses.* The Ronald Press Co., New York, 1950, 77–78.

On culm and leaf sheath of *Alloteropsis cimicina* Stapf. (Gramineæ), Pattambi, 15–6–51, N. V. Sundaram.

The stromata are brownish orange 6–22 mm. long and 2–5 mm. thick. The conidia are ovoid and hyaline. This is a new host for this fungus.

Aecidium meliosmæ-wightiæ sp. nov.

Woody galls formed on twigs, petioles and veins of leaves, galls brown, 5–7 cm. long and 1–3 cm. thick, studded with pycnia and æcia. Pycnia black, subcuticular, hemispherical or conical, 105–145 μ wide and 42–80 μ high, paraphyses lacking. Aecia numerous, deeply immersed in the gall, 700–1,500 μ broad, peridiate, peridial cells polygonal, 51 $\times$23 μ, promiently verrucose; æciospores catenulate, light to deep brown, of different shapes, oblong to angular, sometimes pointed, 28–72 $\times$20–35 μ, wall subhyaline upto 4 μ, thick, prominently verrucose.

Gallæ lignosæ ramis petiolis atque venis foliorum insidentes, brunneæ, 5–7 cm. longæ, 1–3 cm. crassæ, punctatæ pycniis atque æciis. Pycnia nigra, subcuticularia, hemispherica vel conica, 105–145 μ lata, 42–80 μ alta, paraphysibus nullis. Aecia plurima, profunde in gallam immersa, 700–1,500 μ lata, peridiata, cellulis peridialibus polygonalibus 51 $\times$23 μ, prominenter verruculosis; æciosporæ catenulatæ, pallidæ vel profunde brunneæ, multiformes, oblongæ, vel angulares, aliquando acutæ, 28–72 $\times$20–35 μ; parietes subhyalini, ad 4 μ crassi, prominenter verruculosi.

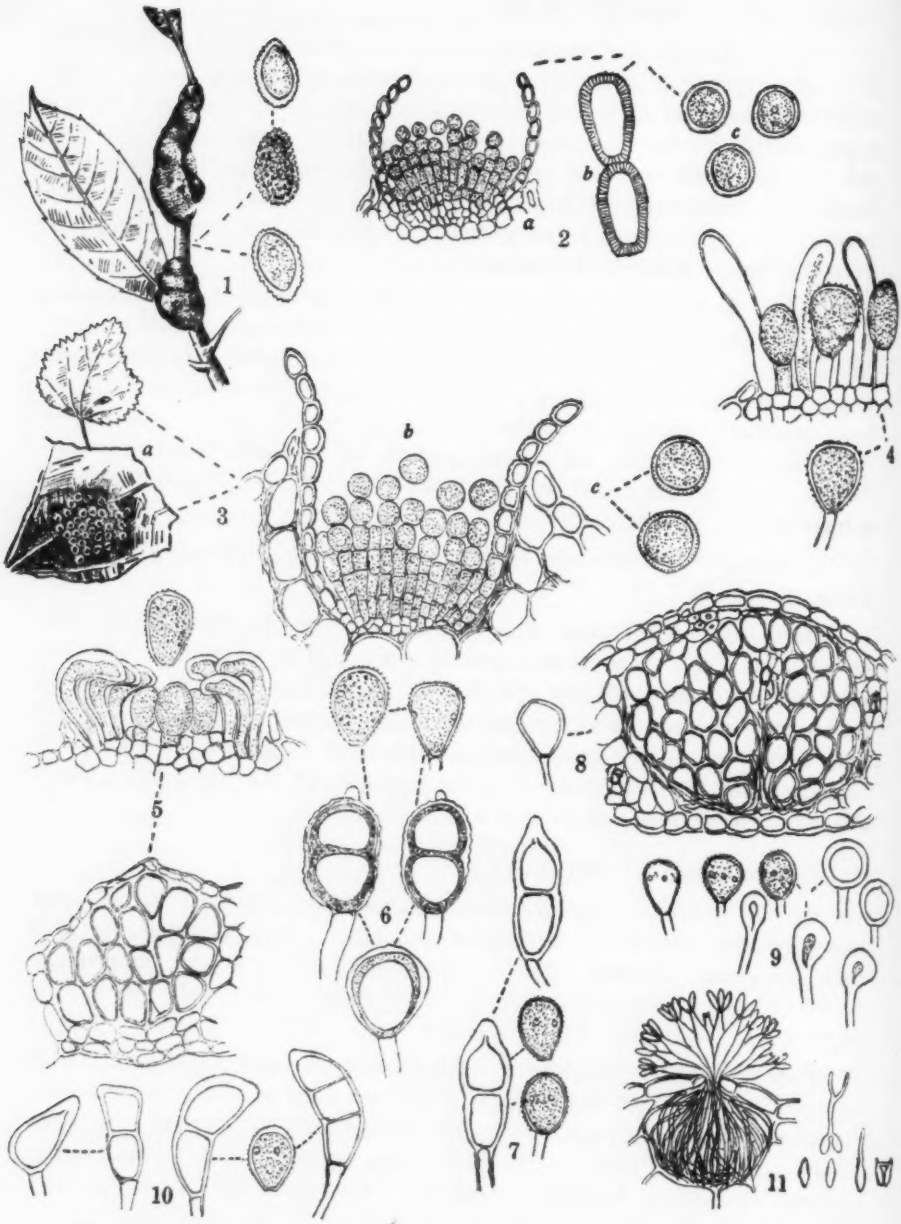
On living twigs petioles and leaves of *Meliosma wightii* Planch. (Sabiaceæ), Ootacamund, 15-7-1951, T. S. Ramakrishnan.

Hard woody galls of varying sizes are seen on the trees. These galls are studded with numerous æcia and pycnia mixed together. The æcia are sunk in the tissues of the gall. On bursting, the frayed edges of the whitish evanescent peridium are seen round the brown contents of the æcia. Older æcia appear as cavities in the gall. Four other species of *Aecidium* have been recorded on this genus. But the formation of the big woody galls is characteristic of this rust.

Aecidium anaphalis-leptophyllæ sp. nov.

Pycnia minute, amphigenous, subepidermal, 46–93 $\times$28–68 μ; æcia hypophyllous, sparse or crowded, 0·3–0·6 mm. high, peridiate, peridial cells polygonal, hyaline, thick-walled, walls with striæ-like thickenings, 34–77 $\times$22–46 μ; æciospores orange yellow, globose, wall hyaline and finely verruculose, catenulate, 25–31 $\times$19–25 μ.

Pycnia minuta, amphigena, subepidermalia, 46–93 $\times$28–68 μ; æcia hypophylla, sparsa vel aggregata, 0·3–0·6 mm. alta, peridiata, cellulis peridialibus polygonalibus, hyalinis, crasso pariete præditis, pariete vero striis instar crassitatum ornato, 34–77 $\times$22–46 μ; æciosporæ aurantiaco-luteæ, globosæ, parietibus hyalinis et minute verruculosis ornatæ, catenulatæ, 25–31 $\times$19–25 μ.



FIGS. 1 to 11.—Fig. 1. Sketch of the galls formed by *Aecidium meliosmae-wightii* (×½); æciospore (×300). Fig. 2. (*a*) Section of æcium of *Aecidium anaphalis-leptophyllæ* (×100); (*b*) peri-

dial cells (×500); *c*, æciospores (×500). Fig. 3. (*a*) Sketch of the leaf of *Pavonia odorata* showin æcial cups of *Aecidium pavoniæ-odoratæ* (magnified); (*b*) section of æcium (×250); (*c*) æciospores (×500). Fig. 4. *Uredo acalyphæ-fruiticosæ*, portion of uredium and one urediospore (×400). Fig. 5 *Phakopsora incompleta*, uredium and telium, (×400). Fig. 6. *Puccinia gymnopetali-wightiæ* urediospores, teliospores and one mesospore (×400). Fig. 7. *Puccinia desertorum*, urediospores and teliospores (×400). Fig. 8. *Uromyces leptodermus*, telium and teliospore (×400). Fig. 9. *Uromyces amphilophis-insculptæ*, urediospores, teliospores and paraphyses (×300). Fig. 10. *Puccinia bulbostylidis*, urediospore, teliospores and mesospore (×400). Fig. 11. *Microstroma delonicis*, acervulus and germinating conidia (× 300).

On living leaves of *Anaphalis leptophylla* DC. (Compositæ), Sholada near Ootacamund, 15–10–1951, T. S. Ramakrishnan.

Since pycnia and æcia were alone observed and no associated telial stage was present this is placed in the form genus.

Aecidium pavoniæ-odoratæ sp. nov.

Pycnia amphigenous, reddish brown, sub-epidermal, globose with a protruding column of paraphyses upto 200 μ long. Aecia hypophyllous, cupulate, clustered, yellow, subepidermal, peridiate, peridial cells hyaline, polygonal, 16–33 ×12–22 μ, strongly verrucose; æciospores catenulate, round, wall subhyaline, finely verruculose, yellowish orange, 19–28 ×16–22 μ.

Pycnia amphigena, rubro-brunnea, subepidermalia, globosa, ornata columna paraphysum exserta usque ad 200 μ longa. Aecia hypophylla, cupulata, aggregata, lutea, subepidermalia, peridiata, cellulis peridialibus hyalinis, polygonalibus, 16–33 ×12–22 μ, fortiter verruculosa; æciosporæ catenulatæ, rotundæ, parietibus subhyalinis, minute verruculosis, luteo-aurantiacæ, 19–28 ×16–22 μ.

On living leaves of *Pavonia odorata*, Willd. (Malvaceæ), Kallar (Coimbatore), 26–1–52, N. V. Sundaram and G. Siddhalinga Reddy.

Pycnia and æcia alone were observed. These stages have not been recorded on this host genus. Hence it is identified as a new species of the form genus.

Puccinia bulbostylidis Doidge.

Doidge, E. M. .. *Bothalia*, 1926, **2**, 117.

On living leaves and peduncle of *Bulbostylis barbata* Kunth. (Cyperaceæ) Pattambi, Malabar, 15–11–1951), N. V. Sundaram.

Two species of *Puccinia* have been described on this host genus, *viz.*, *P. bulbostylidis* and *P. bulbostylidicola*, Thirum. The rust under study has urediospores which are brown, 16–25 ×12–22 μ and with the germpores just above the middle. The telia are black and long covered by the epidermis.

The teliospores are clavate normally 2-celled with the apices pointed or flattened, and thickened upto 6·5 μ. One- and three-celled teliospores are also present. The teliospores measure 31–62 $\times$12–22 μ and have a persistent stalk upto 31μ long and coloured brown. The above characters show that this rust closely resembles *P. bulbostylidis* and is identified as such.

Puccinia desertorum Syd.

Saccardo, P. A. .. *Syll. Fung.*, 1912, **21**, 664–65.

On the leaves of *Evolvulus alsinoides* L. (Convolvulaceæ) Kallar, (Coimbatore), 26-1-52, N. V. Sundaram.

Five species of *Puccinia* have been recorded on this host genus, *viz.*, *P. lithospermi* Ell. and Kellerm., *P. tuyutensis* Speg., *P. enecta* Speg., *P. bellurensis* Thirum. and *P. desertorum* Syd. Of these, the first two are claimed to be synonymous by some, though Jackson (1931) does not accept this. The rust under study has thickened apices (upto 12 μ) for the teliospore. The teliospores measure 31–47 $\times$19–25 μ and urediospores are mixed with the teliospores. The rust most closely resembles *P. desertorum* which has been recorded on the same host from Africa.

Puccinia gymnopetali-wightiæ sp. nov.

Uredia amphigenous, mostly hypophyllous, subepidermal, erumpent, brown; urediospores pedicellate, ovate, elliptic or subglobose, brown, echinulate, wall brown in colour, 4 germpores above the middle, 22–31 $\times$16–24 μ; telia black, amphigenous, mostly hypophyllous, subepidermal; teliospores usually 2-celled elliptic with rounded ends, 28–74 $\times$25–31 μ, wall warty, lamellated, outer layers subhyaline, inner chestnut brown, pedicellate, pedicel hyaline, upto 33 μ long and 10 μ broad, with a distinct knob-like papilla at the apex (upto 3·5 μ) of the spore; mesospores rare, 25–31 $\times$19–25μ with a thick many-layered wall and tapering apex, wall upto 4·3 μ thick.

Uredia amphigena, ut plurimum hypophylla, subepidermalia, erumpentia, brunnea; uredosporæ pedicellatæ, ovatæ, ellipticæ vel subglobosæ, brunneæ, echinulatæ, pariete brunneo, 4 germinationis poris supra medium, 22–31 $\times$16–24 μ; telia nigra, amphigena, ut plurimum hypophylla, subepidermalia; teliosporæ generatim 2-cellulatæ, ellipticæ apicibus rotundis, 28–74 $\times$25–31 μ, pariete verrucoso, lamellatæ, straturæ exteriores subhyalinæ, interiores vero castaneo-brunneæ, pedicellatæ, pediculo hyalino, usque ad 33 μ long., 10 μ latit., papilla tuberosa usque ad 3·5 μ in apice sporæ; mesosporæ raræ, 25–31 $\times$19–25 μ, ornatæ pariete crasso, multis stratis consistente, apice fastigato, pariete usque ad 4·3 μ crasso.

On living leaves of *Gymmopetalum wightii* Arn. (Cucurbitaceæ), Pannaikadu (Palnis), 20–12–51, N. V. Sundaram.

The teliospores resemble those of *Uropyxis* in appearance but the absence of two germpores in each of the telial cells preclude the inclusion of this rust in this genus. It differs from *P. cephalanadræ* Thuem. recorded on *Cephalandra* in having much bigger teliospores. Furthermore the wall has many layers in the rust under study and is warty instead of having fine lines as in the former. It is described as a new species of *Puccinia*.

Puccinia chloridis-incompletæ sp. nov.

Uredia amphigenous, brown, subepidermal, paraphysate with marginal incurved thick walled clavate paraphyses; urediospores subglobose to obovate, yellowish brown, 19–28 ×15–22 μ, echinulate, with 6 distributed germpores. Telia mainly hypophyllous, black, erumpent, oblong, paraphysate, paraphyses 25–46 ×9–13 μ, marginal, incurved with thickened walls, yellowish; teliospores two-celled, chestnut brown, elliptic with rounded ends, apex slightly thickened or not, slightly constricted at the middle, smooth, 25–31 ×19–21 μ, stalk persistent, hyaline to subhyaline, upto 77 μ long, mesospores present, 22–25 ×19–22 μ, apices of mesospores much thickened upto 10 μ.

Uredia amphigena, brunnea, subepidermalia, paraphysata paraphysibus marginalibus, incurvis, clavatis, pariete, crasso præditis; uredosporæ subglobosæ vel obovatæ, luteo-brunneæ, 19–28 ×15–22 μ, echinulatæ, germinationis poris 6 dispersis. Telia ut plurimum hypophylla, nigra, erumpentia, oblonga, paraphysata, paraphysibus 25–46 ×9–13 μ, marginalibus incurvis, luteis, pariete crasso præditis; teliosporæ 2-cellulatæ, castaneo-brunneæ, ellipticæ apicibus rotundis, apice vel tenuiter vel nullo modo incrassato, tenuiter constrictæ ad medium, leves, 25–31 ×19–21 μ, pediculo persistente, hyalino vel subhyalino, ad 77 μ longo; mesosporæ adsunt, 22–25 ×19–22 μ, apicibus valde incrassatis ad 10 μ.

On living leaves of *Chloris incompleta* Roth. (Gramineæ), Kallar (Coimbatore), 26–1–52, N. V. Sundaram.

Two rusts have been recorded on this host genus, *viz.*, *P. chloridis* Speg. and *P. gymnopogonis* Syd. The rust under study differs from these in the possession of paraphyses in the sori. The teliospores resemble those of *P. gymnopogonis* but due to the presence of the urediospores with 6 germpores and the paraphyses, the rust under study is considered to be different.

Puccinia nakanishikii Diet.

Saccardo, P. A. .. *Syll. Fung.*, 1912, **21**, 691.

On living leaves of *Cymbopogon coloratus* Stapf. (Gramineæ), Pannaikadu (Pulney Hills), 20–12–51, N. V. Sundaram.

The sori are linear and both uredia and telia are present.

Uromyces amphilophis-insculptæ sp. nov.

Uredia hypophyllous, brown, linear, subepidermal, erumpent; urediospores obpyriform, ovate or subglobose, reddish brown, echinulate, 23–32 × 17–22 μ, germpores 6, above the middle, paraphyses numerous, capitate, with yellow much thickened wall. Telia hypophyllous, erumpent, black; teliospores one celled, smooth, chestnut brown, 21–30 × 21–26 μ, subglobose, with one pore at the apex, pedicellate, pedicel hyaline or subhyaline upto 30 μ long.

Uredia hypophylla, brunnea, lineraria, subepidermalia, erumpentia; uredosporæ obpyriformes, ovatæ vel subglobosæ, rubro-brunneæ, echinulatæ, 23–32 × 17–22 μ, germinationis poris 6 supra medium, paraphysibus plurimis, capitatis, luteo, valdeque incrassato pariete ornatis. Telia hypophylla, erumpentia, nigra; teliosporæ unicellulatæ, leves, castaneo-brunneæ, 21 × 30 × 21–26 μ subglobosæ, unico poro ad apicem ornatæ, pedicellatæ, pediculo hyalino vel subhyalino usque ad 30 μ longo.

On living leaves of *Amphilophis insculpta* Stapf. (Gramineæ), Musiri (Trichinopoly District), 11–12–1951, T. S. Ramakrishnan.

This rust differs from the other species of *Uromyces* recorded on *Andropogoneæ* in the shape and size of the spores and the presence of paraphyses.

Uromyces leptodermus Syd.

Doidge, E. M. .. *Bothalia*, 1926, **2**, 48.

On living leaves of *Brachiaria ramosa* Stapf. (Gramineæ), Coimbatore, 26–1–52, N. V. Sundaram; on *Panicum antidotale* Retz. (Gramineæ), Coimbatore, 28–1–52, T. S. Ramakrishnan.

Both uredia and telia are present. The telia are deep seated more or less occupying the space between the two epidermal layers. The spores are massed together simulating the sorus of *Phakopsora*. But they are pedicellate. The wall is thin and the spores are of many shapes owing to pressure. The sorus remains covered by the epidermis.

The same rust is present on *Panicum antidotale* also. The uredia on this host are amphigenous, in groups and erumpent. The urediospores

have the triangular appearance in sectional view with a germpore at each angle. The telia are minute, black, in groups mixed with uredia and immersed in the tissues. The spores are pedicellate, brown, thick-walled and without any apical thickening.

Sydow, H. and P. (1910) have described *U. superfluus* Syd. on this host from India. The rust now recorded is not this species but *U. leptodermus*.

Phakopsora incompleta (Syd.) Cumm.

Cummins, G. B. .. *Mycologia*, 1950, **42**, 786–87.

On living leaves of *Ischæmum aristatum*, L., Kallar (Coimbatore), 26–1–52, N. V. Sundaram.

Both uredia and telia were present on the specimens.

Uredo acalyphæ-fruiticosæ sp. nov.

Rust spots minute, brown; uredia hypophyllous, clustered in the spots erumpent, paraphysate, paraphyses clavate, erect or bent, hyaline to subhyaline, 28–77 ×6–16 μ, distributed throughout the sorus; urediospores pedicellate, obovate, subglobose, light brown, minutely echinulate, 24–34 ×15–25 μ, pores not distinct.

Maculæ rubiginosæ minutæ, brunneæ; uredia hypophylla, aggregata in maculis, erumpentia, paraphysata, paraphysibus clavatis, erectis vel curvis, hyalina vel subhyalina, 28–77 ×6–16 μ, distributa per sorum; uredosporæ pedicellatæ, obovatæ, subglobosæ, pallide brunneæ, minute echinulatæ, 24–34 ×15–25 μ, poris haud distinctis.

On living leaves of *Acalypha fruiticosa* Forsk. (Euphorbiaceæ), Kallar (Coimbatore), 26–1–52, N. V. Sundaram.

The uredial stage alone is present. *Phakopsora antiguensis* Cumm. has been recorded on this genus. But the urediospores of the rust under study are pedicellate and the paraphyses are not confined to the margin of the sorus. In *P. antiguensis* the urediospores are sessile and the paraphyses marginal.

Uredo tephrosiæ Rabenh.

Sydow, H. and P. .. *Ann. Mycol.*, 1916, **4**, 257.

On living leaves of *Tephrosia purpurea* Pers. (Papilionatæ), Pattambi (Malabar), 15–9–51, T. S. Ramakrishnan.

The uredial stage alone was prevalent. The Sydows are of the opinion that this may belong to *Ravenelia mitis* Syd. The measurements of the

spores and the presence of the 4 equatorial germ pores bring out the identity of the uredial state now recorded with *U. tephrosiæ*.

Russula lepida Fr.

Saccardo, P. A. .. *Syll. Fung.*, 1910, **19**, 461.

On the ground in the pine forests at Kodaikanal, 15–10–51, E. Amritharaj.

This grows on the ground in the pine forests. The stipe is whitish, firm and 4–6 cm. high. The pileus is of varying shades of violet, convex or depressed in the middle and the gills are whitish and crowded. The spores are hyaline and possess the characteristic markings. This mushroom is edible and is regularly gathered from the forests and immediately cooked or stored for future use. The mushrooms are threaded into strings and hung over the oven smoke for drying for a week before storage. The dried mushrooms are used later locally or sent out to other cities.

Microstroma delonicis sp. nov.

Spots indefinite, yellowish, acervuli hypophyllous, minute, white, aggregated into circular spots upto 2 mm. in diameter, rarely epiphyllous. Conidiophores emerging through stoma, fasciculate, fusoid to lageniform, 16–27 $\times$4–6 μ; sterigmata conical, 2 $\times$1 μ; conidia two to six from each conidiophore, oblong to spindle-shaped, with bluntly rounded ends, hyaline, one-celled, 8–12 $\times$2–4 μ.

Maculæ indefinitæ, flavidæ; acervuli hypophylli, minuti, albi, aggregati sæpe in maculis circularibus ad 2 mm. diam; raro etiam epiphylli; conidiophoræ erumpentia per stoma fasciculatæ, fusoidea vel lageniformia, 16–27 $\times$4–6 μ; sterigmata conica, 2 $\times$1 μ; sporæ binæ vel senæ insidentes singulis conidiophoris, oblongæ vel fusiformes, apicibus hebete rotundatis, hyalinæ, unicellulatæ, 8–12 $\times$2–4 μ.

On living leaves of *Delonix elata* Gamb.(Cæsalpineæ), Bapatla, 17–8–1951, K. V. Srinivasan.

The fungus forms snow white growths on the lower surface of the leaflets. Sometimes defoliation results owing to increased incidence of the disease. The hyphæ collect in the substomatal air space forming fascicles. From these conidiophores originate.

Microstroma albizziæ Syd.

Sydow, H. and P. .. *Ann. Mycol.*, 1914, **12**, 263.

On living leaves of *Albizzia amara* Boiv. (Mimosoidæ), Kallar (Coimbatore), 26–1–52, N. V. Sundaram.

The fungus produces whitish growths on the lower surface of the leaves. The same fungus was collected from the Pulney Hills also.

Pestalotia macrotricha Klebh.

Guba, E. F. .. *Phytopath*, 1929, **19**, 214.

On living leaves of *Rhododendron arboreum* Sm. (Ericaceæ), Ootacamund, 12–12–51, N. V. Sundaram.

Large grey spots are formed on the leaves. The pustules are black, punctiform and amphigenous in the central portions of the spots.

We are grateful to Rev. Dr. H. Santapau of St. Xaviers College, Bombay, for kindly rendering the diagnoses into Latin. Our thanks are also due to the Government Lecturing and Systematic Botanist, Coimbatore, for identifying some of the host plants.

References

Fitzpatrick, H. M. .. *The Lower Fungi—Phycomycetes*. McGraw-Hill Book Co., New York, 1930, 218–19.

Jackson H. S. .. *Mycologia*, 1931, **23**, 497–98.

Sydow, H. and P. .. *Monographia Uredinearum*, 1910, **2**, 337.

720-52 Printed at the Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent
and Published by The Indian Academy of Sciences, Bangalore.

ERRATA

Vol. XXXIII, No. 4, *April* 1951, *Section B*

Page 166, 3rd para, line 10, " action " *should read* " actin ".

Page 175, in the scheme of first para, part D, " without " *should read* " with ".

Page 190, last para, second line, " milk " *should read* " mild ".

ERRATUM

Vol. XXXIV, *No.* 5, *November* 1951, *Section B*

Page 258, Table I, the line which reads " Gr. VII, 5, 120 hours " *should actually read* " Gr. VII, 5, 192 hours ".

ERRATUM

Insert the following as the last paragraph on page 153 of Vol. XXXV, No. 4, Section B:—

ACKNOWLEDGMENT

The above work was carried out in the Institute of Science, Bombay.

OBSERVATIONS ON ERGOTS ON *PENNISETUM* AND OTHER GRASSES

By T. S. Ramakrishnan, F.A.Sc.

(*Government Mycologist, Coimbatore*)

Received April 18, 1952

Ergot has been recorded on over thirty different grasses in South India. On some hosts the sphacelial state alone has been observed. On others sclerotia also have been noticed. More than four species of *Claviceps* are definitely to be found among these, *viz.*, *Claviceps purpurea* Tul., *C. viridis* Pad. and Azm., *C. paspali* Stev. and Hall., and *C. pusilla* Ces. The germination of the sclerotia has been followed in a few but not in all. Therefore the identity of the species could not be determined in the latter. The conidia are small or big, oblong, lunulate or triangular. Based on the characters of the conidia the ergots (whose perithecial state has not been observed) recorded in South India can be grouped into five or six well defined classes. The cold months from October to February with intermittent showers and heavy dew appears to be the most suitable period for large-scale infection of grasses in the plains of South India.

In November 1950 all the plants in a group of allotetraploid hybrids of *Pennisetum typhoides* × *P. purpureum* grown by Dr. N. Krishnaswamy, Cytogenecist, at the Millets Breeding Station, Coimbatore, were heavily infected by an ergot which prevented seed setting. Examination of the ears showed the presence of honeydew and sclerotia in most of the spikelets. The sclerotia were small and dark brown, just projecting from between the lemma and palea. The honey dew was light honey coloured. The conidia were however hyaline and lunulate and measured 18 × 5 μ (13–25 × 3–6). The shape of the conidia suggested affinity to the ergots recorded on *P. hohenackeri*, *Brachiaria ramosa*, *Cenchrus ciliaris*, etc. (Ramakrishnan, 1947). Further studies were carried out with the new fungus and other strains infecting *B. ramosa* and *Urochloa setigera* and the observations are recorded in this communication.

Materials and Methods

The infected ears of the hybrid above mentioned were dried in shade and stored in corked tubes for obtaining the inoculum. Spore suspensions were made in distilled water using two or three infected spikelets for 20 c.c.

of water. The suspension was of a thin milky colour. It was atomised on young ears just when stigmas were protruding. After spray inoculation, the ears were enclosed in glass tubes plugged at the ends with damp cotton-wool. The tubes were removed after three days under humid conditions or kept on for two more days if bright weather prevailed. The analyses of sclerotia were made by the method described by Mukerji and De (1944).

EXPERIMENTAL RESULTS

The spray inoculations were carried out in the months of April to November. In susceptible hosts positive infection took place irrespective of the month, provided the humidity round the ear was maintained as near saturation level as possible.

The results of infection experiments are given below:

TABLE

Statement showing the results of inoculation

Host	Results
Pennisetum typhoides ..	Positive infection with honey dew in 8 days
P. purpureum	" " " " 7–8 "
P. reupellii	" " " " 8 "
P. hohenackeri	" " " " 8 "
P. alopecuros	" " " " 8 "
P. polystachyon	" " " " 8 "
Cenchrus ciliaris	" " " " 7 "
C. setigerus	" " " " 7 "

This ergot infects all the species of *Pennisetum* included in the trials and the species of *Cenchrus*. The inoculations were repeated on different hybrids of *P. purpureum* × *P. typhoides* exhibiting differences in chromosome numbers. Sterile F_1 ($2n = 21$), fertile F_2 ($2n = 22$), allotetraploids ($2n = 28$) and other F_2 ($2n = 42$) populations were included in the trials to note whether any differences in their reaction are noticeable. All of them were readily infected in six to seven days producing copious honey dew.

Both the fertile and the sterile progenies were equally infected showing thereby that the fertility of the grasses is not essential for successful infection.

The course of infection was closely followed in one of the hybrid hosts (*P. typhoides* × *P. purpureum*). The suspension was sprayed on the ear when the stigmas were coming out. Random spikelets were taken out at 24-hour intervals to watch the progress of infection. After 48 hours, germinating spores were found on the surfaces of stigma, style and ovary. After a lapse of 72 hours mycelial growth was evident at the base of the ovary with penetration into the outer layers of cells. The spores on the style and stigma had germinated but there were no signs of further growth or infection of these parts. Ninety-six hours after inoculation the ovary was found to be completely invaded and conidial formation had commenced in some portions near the surface. In five days honeydew was formed. The stamens, style and stigma were not involved. On the sixth and seventh days more profuse mycelial growth took place and the mass thus formed developed conidia from all over the surface borne on closely arranged conidiophores. In ten days sclerotia had developed in the infected spikelets.

The sclerotia were small, dark and grey coloured but white inside. The alkaloid content of the sclerotia was chemically assayed. There were only slight traces of the alkaloid. This would suggest that the possibility of ergot poisoning through the presence of the disease in the grain crop is remote. There are indications however that physiologically different strains of this ergot exist and that there might be differences in host range, alkaloid content and other characters between these.

The ergot under study closely resembles the ones recorded on *P. hohenakeri* and *Cenchrus* spp. (Thomas, *et al.*, 1945). But the sclerotia formed on the former are bigger, while on the latter hosts sclerotia are not formed at all either by the isolates naturally found on them or by those found on *Pennisetum*. Thus the host influences the size and development of the sclerotia. Similar behaviour has been observed in *C. purpurea* by Atanasoff (1926). *C. purpurea* which forms sclerotia on rye and many other hosts infects *Anthoxanthum odoratum* but forms only conidia and not sclerotia. The fungus under study has numerous hosts and differences in the size and formation of the sclerotia, host range, etc., are exhibited by the strains on different hosts. The ergot on *Brachiaria ramosa* has been collected from different places in the State. The conidia are identical in shape and size to those found on *Pennisetum*. The strain from the *Pennisetum* hybrid failed to infect *B. ramosa*. But the strain on *Cenchrus ciliaris* was however able to infect *B. ramosa*.

Thus it seems that there is evidence of differences in host range existing between the forms found on different hosts. It has been stated above that analyses of the small sclerotia formed on *Pennisetum* revealed only traces of the alkaloid. Some samples of the sclerotia formed on *B. ramosa* collected from South Kanara however contained a high percentage of the alkaloid (0·8% calculated as ergotoxine). A sample of this ergot was sent to the Veterinary Investigation Officer, Ranipet, for feeding trials. He has reported that guinea pigs fed with crushed sclerotia mixed with the food died in a couple of days. Another strain with conidia closely resembling those found on *Pennisetum* hybrid was observed to infect the spikelets of *Urochloa setigera* at Kallar (Coimbatore). This strain readily passed on to *Pennisetum* hybrids and developed honeydew and sclerotia in 10 days. The sclerotia obtained from *Urocholoa* contained over 0·5% of alkaloid. But the sclerotia formed on *Pennisetum* hybrids by the same strain had only traces of the alkaloid. It therefore becomes evident that the strain of the fungus on one host has little alkaloid while on another host it develops a high alkaloid content. There is a possibility of cattle poisoning from the strains having a high alkaloid content when cattle are grazed on infected grasses.

The ergot sclerotia did not germinate even after stratification and exposure to low temperature. The question arises as to how the fungus survives from year to year. No specimen or ergot showing germination of sclerotia under natural conditions was observed. Nor did they germinate in the laboratory after various treatments. It was therefore surmised that the survival might be through the viability of the conidia. In order to test this, germination of the conidia from specimens air-dried and stored in closed glass tubes in the laboratory was determined at monthly intervals. At the same time ears of *Pennisetum* hybrids were also inoculated with suspensions of the spores. In hanging drop cultures the spores were found to germinate readily for a period of seven months after collection. In the eighth month germination was poor and there was no germination in the ninth month. But even in the tenth month germination became evident when the spores were floated on drops containing crushed young ovaries of the host. Successful infections were obtained in the ears of the hybrids sprayed with a suspension of spores 13 months old. The above results definitely show that the fungus can survive from year to year through the agency of the conidia. The large number of hosts with different flowering seasons will be of additional advantage in the perpetuation of the ergot. These ergots are common in several districts. Some of them contain high percentages of the alkaloid, ergotoxine. There is thus a danger of stock

poisoning by feeding on infected grasses. The infection is however confined to the ears. There is no danger from grazing on grasses which have not flowered. After flowering it is better to examine the ears for freedom from ergot before letting in the cattle to graze. Otherwise the ears should be cut off so that cattle have no chance of eating ergotized grains.

I am thankful to Sri P. Ramakrishna Pillai, the Veterinary Investigation Officer, for conducting the feeding trials. I am indebted to Dr. N. Krishnaswamy and to Sri. N. V. Sundaram for all the help rendered during the course of these investigations.

References

1. Atanasoff, D. .. *Ergot of Grains and Grasses.* Office of Cereal Investigations, U.S.D.A. (Mimeo.), 1928.
2. Mukerji, B. and De, N. K. *Curr. Sci.*, 1944, **13**, 128.
3. Ramakrishnan, T. S. .. *Proc. Indian Acad. Sci.*, 1947, **26 B**, 139.
4. Thomas, K. M., Ramakrishnan, T. S. and Srinivasan, K. V. *Ibid.*, 1945, **21**, 94.

ASCORBIC ACID AND *FUSARIUM* WILTED PLANTS

By R. Kalyanasundaram

(*University Botany Laboratory, Madras*)

Received July 5, 1952

(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

Introduction

The degree of cellular activity and rate of metabolism of plant tissues has been correlated with concentration of ascorbic acid, those tissues with greatest cellular activity and highest metabolic rate having relatively high ascorbic acid content (Bessey and King, 1933; King, 1936). It is well recognised that chlorophyll-bearing tissues tend to have the highest concentrations of ascorbic acid (Veselkine, *et al.*, 1937), although there is not necessarily a close parallelism between the quantity of chlorophyll and ascorbic acid in any tissue (Randoin, *et al.*, 1936). That ascorbic acid plays an important part in the plant respiratory system is also well known (Szent Gyorgi, 1930).

There appears to be no report so far on the ascorbic acid content of plants infected by fungi in their aerial parts or by soil-borne root infecting fungi causing vascular wilts, although recent work on phytotoxicity of certain antibiotic substances has indicated general dechlorophyllation in wheat by Streptomycin (Wright, 1951). As the first detectable external symptom of wilt diseases is dechlorophyllation (possibly a manifestation of toxemic condition), it seemed to be of interest to follow up the ascorbic acid build up of wilt infected plants under laboratory and field conditions. The organisms and hosts chosen were *Fusarium vasinfectum* on cotton (*Gossypium arboreum* variety K 2) and *Fusarium udum* on red gram (*Cajanus cajan*). Reducing sugar content of wilting leaves was also estimated along with ascorbic acid content since claims and counter claims have been made on its rôle as the immediate precursor of ascorbic acid (Ray, 1934; Guha and Ghosh, 1935; Hawthorne and Harrison, 1937).

Materials and Methods

Cotton plants were grown in sand-culture and were inoculated with the pathogen in the form of mycelial mats. The uninoculated series served as the control. Red gram plants were grown in the laboratory garden soil which is known to produce wilt of red gram every year. Weekly estimations of ascorbic acid were conducted on the leaves of cotton and red gram following Indo-phenol Xylene extraction method (Robinson and Stotz, 1945).

Reducing sugar content of the leaves was also estimated following a standard method (Hagedorn and Jensen, 1923).

Results

Results given in the accompanying Tables I and II indicate that these vascular wilts brought about a striking reduction in the ascorbic acid content of the leaves of both hosts and also an increase in the reducing sugar content.

TABLE I. *Cotton*

Age of plant (in days)	Ascorbic acid (mg./100 g) Fresh weight (mean values)		Reducing sugar (mg. %) Dry weight (mean values)	
	Inoculated	Uninoculated	Inoculated	Uninoculated
10	46·82	31·30	3·48	3·23
17	73·87	64·20	3·76	3·85
*24	96·70	91·80	5·39	3·79
*31	88·80	97·85	5·59	4·14
38	72·60	103·85	5·37	4·47

* Severe wilt symptoms were seen during this period.

TABLE II. *Red Gram*

Age of plant (in days)	Ascorbic acid (mg./100 g.) Fresh weight (mean values)		Reducing sugar (mg. %) Dry weight (mean values)	
	Diseased	Healthy	Diseased	Healthy
*50	183·0	221·0	7·62	4·75
*57	173·2	216·8	9·30	5·15
64	151·6	228·8	7·30	5·46
71	150·8	180·5	6·45	5·14
78	144·6	210·2	5·40	4·85
85	130·4	161·0	7·60	6·19

* Wilt symptoms were seen between the sixth and eighth weeks after planting seedlings.

Discussion

Since ascorbic acid seems to play an important rôle in the overall growth process of plants and animals (King, 1938) and as it is closely associated

with the chlorophyllous tissues, it is reasonable to conclude from the above results that dechlorophyllation and retarded growth, which seem to precede the actual wilting of plants, cause a decrease in the ascorbic acid content. The higher ascorbic acid content of the cotton plants of inoculated series before wilting may be due to an increased metabolic activity of the plant in an attempt to resist the disease in the early stages. The higher reducing sugar content of the inoculated and the diseased plants over the normal ones is of particular interest if we assume that the reducing sugars are the precursors of ascorbic acid. There is presumably a breakdown in the normal metabolism of wilt-affected plants, especially the photosynthetic activity and there is reason to assume that this breakdown in the normal metabolism starts far in advance of the actual wilting of plants. Further work on these lines are being continued to find out the exact nature of host metabolism connected with these vascular wilts.

ACKNOWLEDGEMENTS

The author is greatly indebted to Professor T. S. Sadasivan for his guidance and help.

REFERENCES

Bessey, O. A. and King, C. G. .. "The distribution of Vitamin C in plant and animal tissues and its determination," *J. biol. Chem.*, 1933, **103**, 687.

Guha, B. C. and Ghosh, A. R. .. "Biological Synthesis of Ascorbic Acid," *Nature*, 1935, **135**, 871.

Hagedorn, H. C. and Jensen, B. N. "Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid," *Biochem. Z.*, 1923, **135**, 46.

Hawthorne, J. R. and Harrison, D. C. "Mannose as precursor of Ascorbic acid," *Chem. Abstr.*, 1937, **31**, 7953.

King, C. G. .. "Distribution of Vitamin C in animal tissues," *Physiol., Rev.*, 1936, **16** (2), 238.

——— .. "Physiology of Vitamin C," *J. Amer. med. Ass.*, 1938, **111**, 1098.

Randoin, L., Giroud, A. and Leblond, C. P. "Relation between the Vitamin C contents of various plant tissues and the presence or absence of chlorophyll," *Chem. Abstr.*, 1936, **30**, 751.

Ray, S. N. .. "On the nature of the precursor of Vitamin C in the vegetable kingdom, I. Vitamin C in the growing pea seedlings," *Biochem. J.*, 1934, **28**, 996.

Robinson, W. B. and Stotz, E. .. "The Indophenol Xylene extraction method for ascorbic acid and modifications for interfering substances," *J. biol. Chem.*, 1945. **160**, 217.

Szent Gyorgi, A. .. "On the mechanism of biological oxidation," *Science*, 1930, **72**, 125.

Veselkine, N. V., Lubimenko, V, N., Boulgakova, Z. P. and Ilyine, N. S. "The Action of light on the synthesis of vitamin C," *Chem. Abstr.*, 1937, **31**, 6696.

Wright, J. M. .. "Phytotoxic Effects of some antibiotics," *Ann. Bot, N. S.*, 1951, **15**, 493.

OBSERVATIONS ON THE FOOD AND FEEDING OF THE INDIAN MACKEREL, *RASTRELLIGER CANAGURTA* (CUVIER)*

By B. S. Bhimachar, F.A.Sc. and P. C. George
(*Central Marine Fisheries Research Sub-Station, Calicut*)

Received July 22, 1952

The present communication relates to observations made on the food and feeding of the Indian mackerel, *Rastrelliger canagurta* (Cuvier), which occupies a high rank amongst the food fishes of India and enjoys a wide distribution in the Indo-Pacific area. Except for a few brief notes on the nature of the food by Devanesan and John (1940), John and Menon (1942), Chidambaram (1944) and Chacko (1949) there is no detailed account especially of the seasonal fluctuations in the food elements and the feeding intensity. In a recent paper Chidambaram and others (1952) have given an account of the fat variations and certain biological aspects of the mackerel. The problem of the food and feeding of the European mackerel, *Scomber scombrus* has been studied by Allen (1897 and 1909), Bullen (1908), Ehrenbaum (1914), Steven (1949) and Sette (1950). The present study is only a preliminary investigation and it is hoped that this may form the basis for a more comprehensive work on the subject.

Our thanks are due to Dr. N. K. Panikkar for going through the manuscript and offering helpful suggestions.

Material and Methods

This study is based on biweekly examination of mackerel landed during January 1949 to January 1950. The species were obtained partly from the departmental collections and partly from the commercial catches made in the 6–10 fathom area near Calicut. During 1950 mackerels were examined once in a fortnight and this was helpful in verifying the results obtained during 1949. A total of 395 specimens were examined of which 321 were adults and 74 juveniles. During the period of this investigation mackerels were available near about Calicut practically throughout the year, though the peak season of its fishery was from September to December. The data obtained as a result of the study of triweekly plankton collections made at

* Published with the permission of the Chief Research Officer, Central Marine Fisheries Research Station, Mandapam Camp.

this research station from the 8 fathom area have been used to compare the food environment of the mackerel with its food constituents as seen from the examination of the stomach contents.

The size of the fish[1] and its gonad condition were noted with a view to finding if any correlation existed between these factors and the intensity of feeding of the fish. The stomach was carefully removed, was split open and the contents washed with water into a petri dish. The displacement volume of the total stomach content was determined in each case by making up the volume to 10 ml. and filtering it through No. 60 bolting silk. The difference between the initial volume and the filtrate was recorded as the volume of the gut content. One ml. of the made-up gut content, which was well stirred up, was taken by means of a graduated pipette and spread over a counting slide. This material was examined under a binocular microscope and the number of each species of organism was counted and recorded.[2] The rest of the gut content was preserved in 5% formalin for further study. The food organisms were, in most cases, very well preserved facilitating easy identification, often up to species. The non-identifiable fractions were almost always negligible.

In determining the relative importance of the various groups of organisms constituting the food of the mackerel two methods were followed: (1) the *number method* and (2) the *points method* (Hynes, 1950). It was found that the number method which gives the total number of each type of food organism in the stomach content does not really express the percentage volume of the various organisms in the total food, as there are wide differences in sizes between various food organisms. For instance, some of the diatoms are very small compared to the size of a copepod. The determination of the volumetric percentage value of the different food elements has been attempted by the points method. Hynes (1950) in his review of methods used in studies of the food of fishes has described the method of estimating the percentage composition of various organisms in the food of fishes by the points method as followed by Swynnerton and Worthington. In this method, the percentage composition is calculated by allotting points to the various organisms based on their relative sizes as determined roughly under a binocular microscope by visual estimation. The points so allotted are then tabulated and the percentages calculated. After careful practice for sometime it is possible to calculate the percentage values with a fair degree of accuracy.

[1] Total length from the tip of the snout to the end of dorsal fluke of the caudal fin when it is brought in alignment with the long axis of the body, has been taken.

[2] In case of certain phytoplankton forms each colony or chain is reckoned as a unit.

An Account of the Food and Feeding

The food of the mackerel was composed mainly of copepods, cladocerans, diatoms, peridinians and larvæ and adults of decapods. Among the lesser elements were the appendicularians, polychæte larvæ, post-larval bivalves, pteropods, cirripede nauplii and fish eggs and larvæ. On a few occasions sand grains and fish scales were noticed in the stomach content. The mackerel was observed to feed exclusively on planktonic organisms.

Fig. 1 shows variations in the average number of individuals of different major groups of organisms forming the food of the mackerel during different periods of the year as determined by the number method. A brief account of the different groups of food elements is given below.

Copepoda.—Copepods formed the dominant element in the gut content of the mackerel and they were present in the gut practically throughout the year. Copepod eggs and nauplii were common food elements in February and again in September and October. The maximum feeding on copepods was from January to May. During the monsoon months, June and July, the copepod fraction of the food was low, but increased again during October, November and December. The copepods in the stomachs were invariably in good state of preservation facilitating easy identification. *Paracalanus*, *Oithona*, *Acartia* and *Temora* were the dominant genera forming the food of the mackerel. *Centropages*, *Pseudodiaptomus*, *Corycæus*, *Macrosetella*, *Euterpina* and *Labidocera* and other genera were also observed but in lesser numbers. The following were the common species.

Paracalanus parvus (Claus)
Oithona plumifera Baird
Oithona rigida Giesbrecht
Acartia erythræa Giesbrecht
Temora turbinata (Dana)
Centropages furcatus (Dana)
Pseudodiaptomus sp.
Corycæus sp.
Macrosetella gracilis (Dana)
Euterpina acutifrons (Dana)
Labidocera acuta (Dana)

Cladocera.—Next in importance to copepods as food of the mackerel were the cladocerans. While copepods were observed in the stomach almost throughout the year the cladocerans formed a substantial part of the diet during the period from August to December. It is during this period that they occurred abundantly in the plankton. They were in moderate numbers from January to March. Though only two species, *Evadne tergestina* Claus and *Penilia avirostris* Dana have been known to occur along this coast, they formed important planktonic food on account of their numerical abundance. *Evadne* started coming up in the plankton in the

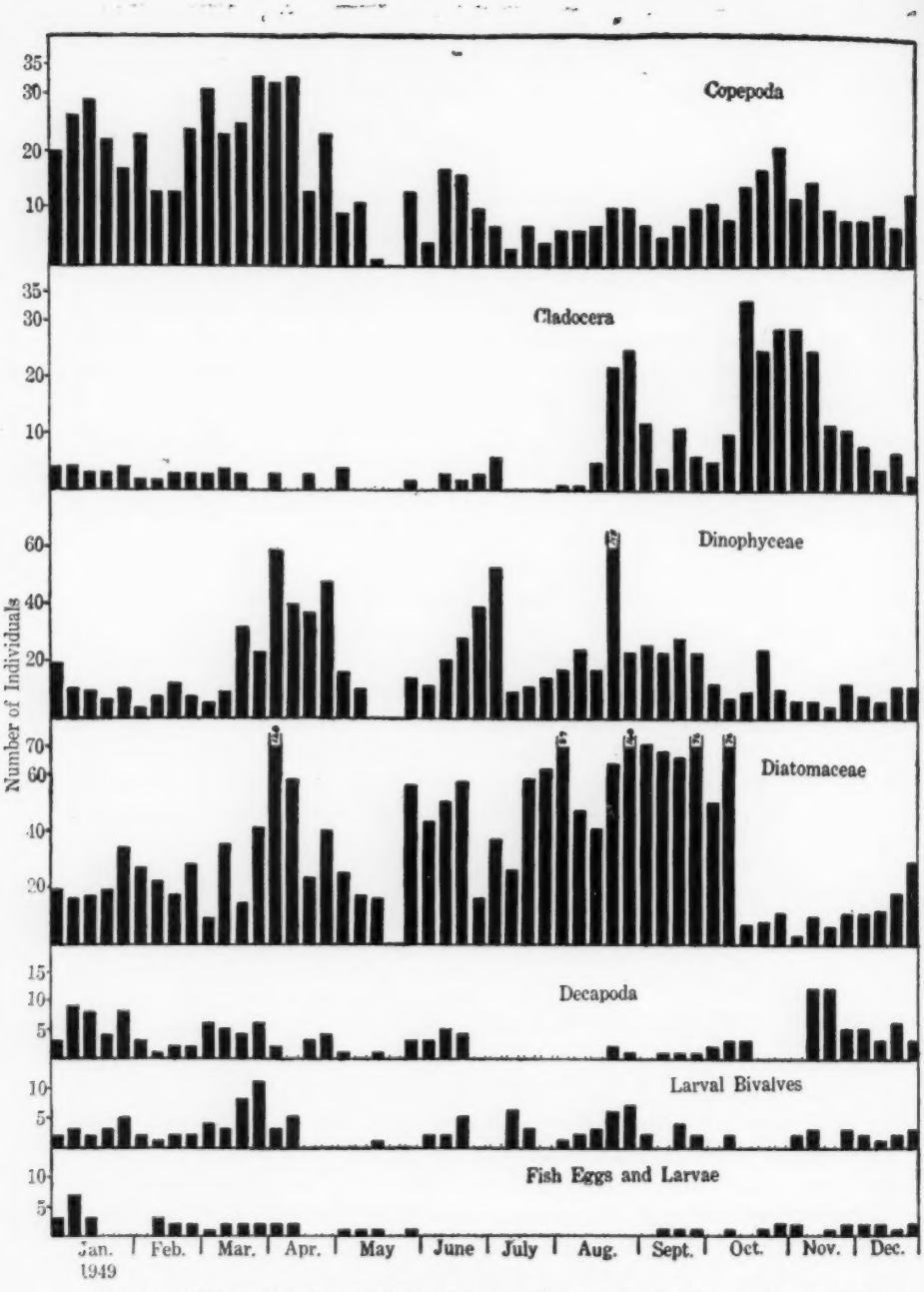


FIG. 1. Variations in the numerical abundance of the major groups of food organisms in 1/10 part of the stomach content of a mackerel.

early part of the monsoon period and was followed by *Penilia* and both the species were dominant during the post-monsoon period. The cladocerans together with the copepods formed the main food of the mackerel during the mackerel season from October to January.

Decapod crustacea.—Larval and adult decapods were present in fair numbers in the stomach contents throughout the year except during the monsoon months of June and July. Larval stages of Penæids, *Acetes* and larval and adult *Lucifer* formed the main decapod elements, contributing to a good percentage of the food of the mackerel from January to April and again in November and December. Zœa and mysis stages of *Acetes* and *Lucifer* were common during the earlier months of the year while eggs and zœa of *Metapenæus dobsoni* were mainly responsible for the high percentage of the decapod fraction in the food in the latter half of November. Adult specimens of *Lucifer* were observed in the food in small numbers throughout the year. Brachyuran zœa, mostly of Portunids along with small numbers of zœa of *Emerita* were also observed in the stomach of the mackerel during summer months.

Dinophyceæ.—These were found to occur almost throughout the year in the mackerel stomach although they indicated definite peak periods correlated with their abundance in the plankton. There was a minor peak in April when species of *Dinophysis* and *Peridinium* dominated, and a major one from June to September when practically all forms of Dinophyceæ occurred abundantly. The following forms represent the dominant species of Dinophyceæ in the food of the mackerel.

Prorocentrum micans Ehrenberg
Dinophysis caudata Saville-Kent
Ornithocercus magnificus Stein
Pyrophacus horologicum Stein
Peridinium conicum (Gran) Ostenfeld et Schmidt
P. depressum Bailey
Ceratium furca (Ehrenberg) Claparede et Lachmann
C. fusus (Ehrenberg) Dujardin
C. massiliense (Gourret) Jorgensen

Bacillariophyceæ (*Diatomaceæ*).—Mackerel feeds actively on diatoms during the latter half of south-west monsoon when the latter occur in great abundance. During the period from January to June the diatom fraction in the food of the mackerel was not appreciable but for a small rise in April due to the occurrence of large numbers of the species of *Pleurosigma* and *Coscinodiscus*. From July to October the general phytoplankton fraction

of the mackerel food was high with a peak in September. Species of *Fragilaria*, *Thallassiothrix*, *Nitzschia* and *Coscinodiscus* contributed to the bulk of the mackerel food during this period. *Biddulphia* and *Rhizosolenia* occurred in moderate numbers almost throughout the year. The following were the main species of diatoms contributing to the food of the mackerel.

Coscinodiscus lineatus Ehrenberg
C. gigas Ehrenberg var. *prætexta* (Janish) Hustedt.
Planktoniella Sol (Wallich) Schutt
Lauderia annulata Cleve
Rhizosolenia robusta Norman
R. setigera Brightwell
Bacteriastrum varians Lauder
Chætoceros coarctatus Lauder
Ch. curvisetus Cleve
Ditylum Sol Grunow
Triceratium favus Ehrenberg
Biddulphia sinensis Greville
B. mobiliensis Bailey
Hemidiscus Hardmannianus (Greville) Mann
Fragilaria oceanica (Cleve)
Thallassiothrix Frauenfeldii Grunow
Asterionella japonica Cleve
Pleurosigma Normanii Ralfs
P. directum Grunow var. *membranacea* Subrahmanyan
Nitzschia sigma (Kutzing) W. Smith var. *indica* Karsten
N. seriata Cleve

Miscellaneous groups.—The minor elements of mackerel food were composed of larval bivalves, polychæte larvæ, chætognaths, appendicularians, larval cirripedes, pteropods, blue green algæ and fish eggs and larvæ. Lamellibranch post-larvæ were found to occur in fair numbers in the stomach of the mackerel in March and again during July and August. There were, however, a few occasions when the fish were observed to have fed almost exclusively on the lamellibranch post-larvæ. Larval stages of *Mytilus* formed the bulk of the larval bivalves. The polychætes observed in the food were mostly larval spionids and were, more or less, limited to the latter half of the south-west monsoon period. *Prionospio* sp. was the most common polychæte occurring during the year. Chætognaths were noticed in the diet on a few occasions although they were present in the plankton in appreciable numbers all through the year. Though eleven species of chætognaths have been known to occur along this coast only two

species, *Sagitta bedoti* and *Krohnitta pacifica* were encountered in the food of the mackerel and that in very small numbers. That the chætognaths are not favoured as food elements by the mackerel is evidenced by the fact that their number in the stomach content was negligible even during the period from October to December when their population was high in the coastal waters (George, 1952). The appendicularian, *Oikopleura* sp. was present in small numbers throughout the year, invariably with the tail severed from the head and occasionally *Fritillaria* was also present. *Pteropods*, particularly *Creseis* sp. were observed in fair numbers during the hot months. Cirripede nauplii were common from September to November and the cypris stage was also occasionally present. The blue-green algæ *Oscillatoria erythraeum* and *O. thiebauti* were only nominally found in the stomach although they were present in plankton in swarming condition during the south-west monsoon period. Fish scales and sand grains were met with in the stomachs in small proportion during the post-monsoon period.

The fish eggs and larvæ were observed occasionally in small numbers during the periods from January to April and September to December and they formed only a negligible part of the diet. The eggs mostly belonged to the species of *Caranx*, *Anchoviella* and *Kowala coval*. A few larvæ of sciænids, clupeids and carangids in their early larval stages, were also observed.

Percentage Composition of Various Food Elements

The volumetric percentage composition of the various groups of food organisms during the different months and the average percentage of each group for the year as determined by the points method is given in Table I.

It is admitted that the determination of the percentage composition of food organisms by the *points method* is arbitrary and that it provides only a rough estimate. Nevertheless, in the absence of a more accurate method, it gives a fair idea of the relative importance of the various food organisms. About 50% of the food of the mackerel was composed of copepods. The other main groups constituted 16% cladocerans, 12% larval and adult decapods, 11% phytoplankton forms, 5·5% lamellibranch larvæ and 4% fish eggs and larvæ. The monthly variations in the percentage values of the various groups can be seen in Table I. There was a close similarity between the values obtained by the number and points methods in respect of the seasonal variations in the abundance of the different groups of food elements (Fig. 1 and Table I).

TABLE I

Percentage composition of the food elements assessed by points method

Food organisms	Jan. 1949	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Average percentage for the year
Phytoplankton	4·0	5·0	4·5	14·0	12·0	13·0	27·0	21·0	20·0	5·0	2·0	5·0	11·0
Copepods	55·0	68·0	62·0	67·40	61·0	52·0	46·3	32·3	33·0	43·0	31·5	39·0	49·6
Cladocerans	5·7	6·3	3·6	2·8	8·0	6·0	9·0	30·6	27·0	40·0	36·0	17·0	16·0
Decapods	21·2	10·5	15·0	8·0	9·0	18·0	..	3·4	5·0	5·0	26·0	24·0	12·0
Fish eggs and larvæ	8·5	7·0	4·5	2·8	8·0	.	..	..	5·2	3·0	0·9	7·5	4·0
Lamellibranch larvæ	5·0	1·6	9·0	3·6	0·8	6·2	13·3	10·5	6·5	1·0	1·8	5·7	5·5
Other forms	0·6	1·6	1·4	1·4	1·2	4·8	4·4	2·2	3·3	3·0	1·8	1·8	1·9

VOLUME OF FOOD

The total volume of food in the stomach ranged from ·7 c.c. in August to 3·5 c.c. by the end of October. The feeding was good from January to May with an average volume of 2·1 c.c. The food volume came down to 1·5 c.c. during the period from May to August indicating poor feeding. The feeding was maximum from September to December when the average volume of food taken was 2·8 c.c. At no period of the year was there complete stoppage of feeding. Only two instances of almost empty stomachs were observed during the year. Two specimens in September and three in October were noticed with very little food in their stomachs, but other specimens examined on the same dates had normally fed. Fig. 2 indicates fluctuations in the extent of feeding, total volume of standard plankton samples taken from the inshore area near Calicut and the maturity stages of mackerel during different periods of the year.

GENERAL OBSERVATIONS AND DISCUSSION

Size and maturity condition of the mackerels and their relation to feeding.— The specimens obtained during January and February had a size range of 20 to 21·6 cm. and were immature with gonad condition not beyond Stage II.* By the latter half of March the specimens showed a more advanced

* The gonad stages referred to in this paper are after the maturity scales adopted by the International Council for the Exploration of the Sea.

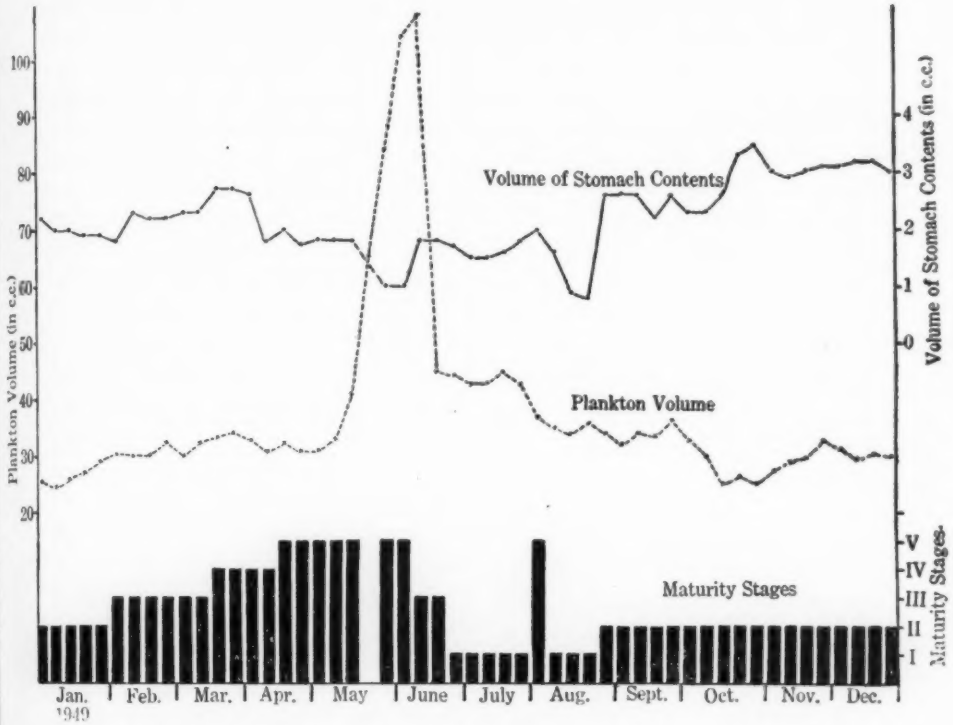


FIG. 2. Variations in the total volume of stomach content, total volumes of standard plankton samples and maturity stages of the mackerel.

gonad condition. In April the gonads were found to occupy half to two-thirds the body cavity and the eggs were still opaque. The size range of the fish was, more or less, the same as that of the previous months. Mackerel was rare during May but the few specimens obtained had a size range of 21·9 to 23·3 cm. and were fully mature reaching Stage V. The gonads filled practically the whole body cavity. In June the gonads showed further advanced condition with a few large transparent ova, a condition more or less similar to that in the ripe ovary of the European mackerel *Scomber scombrus* (Steven, 1950). During May and June, the specimens were very rare. These spawners, it is presumed, would have retreated to the offshore spawning grounds. The absence of mackerel eggs and larvæ in our inshore collections confirms this view. In the month of July there was recruitment of smaller size group, 10 to 13 cm. into the inshore area. They showed gonads which had hardly differentiated and even recognition of sexes was

difficult. During August still smaller size group from 5·6 to 8 cm. was abundantly caught. Only once during August a shoal of mackerel of 20·4 cm. size with gonads in Stage V was caught. This indicates that the mackerel have probably a prolonged spawning period. From September there was a steady increase in mackerel catches and continued to be so till the end of November after which there was a decline in the fishery during the year of study. October and November were peak months for the mackerel fishery. Majority of specimens ranged from 16 to 20 cm. size and were immature with gonad in Stage II. Whether these different size groups of mackerel caught in the coastal waters during different periods of the year belong to a single stock of mackerel in the sea or represent distinct stocks or contingents (Sette, 1950) has yet to be investigated.

There appears to be a distinct correlation between the maturity of the fish and its feeding intensity. From February to the middle of April when the mackerel were maturing, the feeding intensity was high. From the middle of April to June when the fish was mature with advanced gonad condition in Stage V the feeding was observed to be low. During July-August there was recruitment of juvenile sizes into the coastal waters and the feeding was also comparatively poor. Observation that the fish feeds less when they are in advanced stage of maturity during the spawning season is in agreement with the findings of other workers. Jesperson (1928) points out that " Herring feed as a rule to a slight extent immediately before and during spawning " and Ritchie (1937) states in regard to the feeding habits of the haddock that " An analysis of the volumetric data shows that, on an average, the intensity of feeding is much reduced during the spawning season ". Again during the post-monsoon period from September to December when the mackerel population was composed of immature fish the feeding intensity was comparatively high. Venkataraman and Chari (1951) and Chidambaram, *et al.* (1952) have shown that there are two peak periods in a year for fat formation in the mackerel, one in April-May and another in October-November and they attribute it to feeding activity. The period, February-March-April was characterised by abundance of copepods and decapods and to some extent, fish eggs and larvæ and the period, October-November-December was marked by the dominance of cladocerans, decapods and copepods. The high percentage of fat formation by the mackerel during these two periods is perhaps due to its intense feeding on these zooplankton forms.

An examination of the gut contents of a large number of young mackerel of sizes from 5·5 to 7·5 cm. during August revealed that the food of the young mackerel does not radically differ from that of the adult.

Correlation between mackerel food and composition of the plankton.— A close similarity between the food constituents and the planktonic elements in the coastal fishing ground was noticed during different seasons of the year except for the absence of certain groups of organisms in the food though they occurred in the plankton at the time. During the period of intense phytoplankton production especially in the latter half of the south-west monsoon period from July to September the mackerel food showed corresponding dominance in diatoms and peridinians (Table I). Similarly copepods which occurred in large numbers from January to May and again from September to December and cladocerans from August to December and decapods in January and November-December showed corresponding dominance in the food during the respective periods. The other minor forms such as larval bivalves, fish eggs and larvæ, pteropods and appendicularians occurred in the food, more or less, in the same proportion in which they were present in the plankton during different periods of the year. A significant point noticed in this connection was the complete absence of noctiluca from the stomach content of the mackerel though it occurred very abundantly in the plankton from August to October, except in a single specimen in August in which six noctilucæ were present in the stomach. Further, forms like salps, medusæ, ctenophores, stomatopod larvæ, chætognaths and blue-green algæ, though present in the stomach in negligible numbers, were never represented in the food in the same proportions in which they were present in the plankton. These organisms seem to be avoided by the mackerel. To this extent the mackerel does not feed indiscriminately on the plankton, but seems to discriminate edible from the non-edible elements. As to the exact manner by which the non-edible portion of the plankton is avoided is not clearly known. Practically all the non-edible elements especially noctilucæ, are surface living forms and it is possible that the fish, at the regions of their concentrations, prefer to feed below the surface layers of water. On several occasions when the noctiluca was abundant in the plankton it has been observed that the food of the mackerel, as seen from examination of the stomach contents, matched more with the bottom than the surface plankton. The intense concentration of mackerel in the coastal waters from September to January may perhaps be due to the richness of the plankton during these months.

There is close correlation between the organisms in the gut contents of mackerel and those occurring in the plankton collections taken from the 8 fathom zone, both in respect of species and their relative abundance, indicating that they had presumably fed in the inshore area. The food organisms were invariably in fresh condition when the guts were opened for examina-

V 36 1952 XUM

tion. On the other hand, there was considerable variation between the organisms in the gut content of the mackerel and those in the plankton collections taken from the 20 fathom area.

Food Environment.—From a combined study of both the food of the mackerel by the examination of the stomach contents and the plankton collections made near about Calicut it has been possible to assess to some extent the nature and composition of the food environment of the mackerel in the coastal waters. Owing to absence of facilities for offshore work it has not been possible, to examine the nature of the food environment in the offshore area and determine how far the migrations of the mackerel are governed by the food factor. We have at present no knowledge of either the wanderings of the mackerel in the offshore area or the feeding conditions there. Plankton food was available in the inshore area throughout the year though there were seasonal fluctuations in its abundance and composition. During the period from January to May the non-edible elements such as medusæ, ctenophores, chætognaths, salps and other forms appeared in large numbers but a good percentage of the plankton was composed of copepods and decapod larvæ which formed the main food of the mackerel during these months. Immediately after the commencement of the south-west monsoon in the third week of May there was a radical change in the physico-chemical condition of the inshore area. The plankton volume increased as a result of abrupt and abundant production of diatoms, dinoflagellates and other forms. The zooplankton fraction during this period was low. The phyto-plankton bloom declined by the third week of June. From August to December there was a reduction in the phytoplankton population with a corresponding increase in forms of zooplankton in which the cladocerans and copepods dominated. These months represent peak period for the mackerel fishery along this coast and further, intense feeding was noticed among mackerel during the period. In addition to mackerel, other plankton feeding species of fishes were also abundant during the period, bringing about a severe competition for food. The slightly lower plankton volume observed in October and November (Fig. 2) is perhaps indicative of the intense grazing on the rich plankton by the plankton feeding fishes occurring abundantly during the period in the coastal waters. A similar conclusion has been arrived at by Kow (1950) in regard to the feeding relationship of the fishes of Singapore Strait.

From the account of the feeding habit given above, it is obvious that there is no period of the year when mackerel may be said to abstain from food. Similarly, there is also no time of the year when the planktonic organisms which form the food of the mackerel are altogether absent. In

the temperate waters on the other hand, there are very steep seasonal cycles in plankton production and in the consumption of food by plankton feeding fishes, which is reflected in the occurrence of feeding and non-feeding phases in their lives. The annual growth cycle and the formation of winter rings on the scales and otoliths of certain fishes are usually attributed to the feeding cycle. It is reasonable to expect that such growth checks would be less pronounced in tropical fishes feeding on plankton available throughout the year.

Summary

The study of the food and feeding of the mackerel, *Rastrelliger canagurta* was based on the periodical examination of stomach contents of the mackerel and the plankton of the coastal waters near Calicut during the years 1949 and 1950. The relative importance of various food elements have been determined by the *number* and the *points* methods.

The mackerel has been observed to feed almost exclusively on plankton organisms. The main food constituents were copepods, cladocerans, larval and adult decapods, peridinians and diatoms. Post-larval bivalves, fish eggs and larvæ, polychæte larvæ, cirripede nauplii, appendicularians and pteropods were minor elements in the food. There was no appreciable difference between the food of the young and that of the adult mackerels.

The composition of the food varied from season to season depending upon the fluctuations in the occurrence of various planktonic elements. There was a close correlation between the food constituents and the planktonic organisms of the inshore area.

Non-edible forms like salps, medusæ, ctenophores, stomatopod larvæ, chætognaths and noctilucæ were avoided and to that extent there was selective feeding. There was feeding throughout the year although the feeding intensity varied from season to season. There was no marked period of fasting.

The feeding intensity was low during the prespawning and spawning periods and comparatively high in the 16 to 20 cm. size group. It was maximum during the period from September to December when edible plankton was abundant.

References

Allen, E. J. .. "Report on the present state of knowledge with regard to the habits and migrations of the mackerel (*Scomber scomber*)," *Journ. Mar. Biol. Assoc.*, 1897, **5**.

———— .. "Mackerel and Sunshine," *ibid.*, 1909. **8**.

Bhimachar, B. S. and George, P. C. "Abrupt Set-backs in the Fisheries of the Malabar and Kanara Coasts and the Red Water Phenomenon as Their Probable Cause," *Proc. Ind. Acad. Sci.*, 1950, **31**, No. 6, 339-50.

UNIVERSITY OF MICHIGAN LIBRARIES
V 36 1 9 5 2
XUM

Bullen, G. E. .. "Some Notes on the Feeding Habits of Mackerels and Certain Clupeids in the English Channel," *Journ. Mar. Biol. Assoc.*, 1912, **9**, 394–403.

Chacko, P. I. .. "Food and Feeding Habits of the Fishes of Gulf of Manaar," *Proc. Ind. Acad. Sci.*, 1949, **29**, No. 3, 83–97.

Chidambaram, K. .. "Food of the Indian Mackeral (*Rastrelliger kanagurta* Russell) of the West Coast of Madras Presidency," *Curr. Sci.*, 1944, **13**, 214–15.

———, Krishnamurthy, C. G., Venkataraman, R. and Chari, S. T. "Studies of Mackerel—Fat Variations and Certain Biological Aspects," *Proc. Ind. Acad. Sci.*, 1952, **35**, 43–68.

Devanesan, D. W. and John, V. "On the Natural History of *Rastrelliger kanagurta* (Russell) with Special Reference to its Spawning Season and Eggs," *Curr. Sci.*, 1940, **9**.

Ehrenbaum, E. .. "The Mackerel—spawning, larval and post-larval forms, age groups, food, enemies," *Cons. Perm. Inst. Expl. Mer. Rapp. Proc. Verb.*, 1923, **30**, 1–39.

George, P. C. .. "A systematic account of the chætognatha of the Indian coastal waters, with observations on their seasonal fluctuations along the Malabar coast," *Proc. Nat. Inst. Sci. India* (in press), 1952.

Hardy, A. C. .. "The Herring in Relation to its Animate Environment," *Fish Invest. Ministry of Agri. & Fisheries*, Series II, 1924, **7**, No. 1.

Hynes, H. B. N. .. "The food of freshwater Sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*) with a review on methods used in studies of the food of fishes," *Jour. Ani. Ecol.*, 1950, **19**, No. 1, 35–58.

Jespersen, P. .. "Investigations on the Food of the Herring in Danish Waters," *Med. Fra. Komm. for Havundersogelser*, 1928, **2**, No. 2, 1–143.

John, C. C. and Menon, M. A. S. .. "Food and Feeding Habits of Oil Sardine and Mackerel," *Curr. Sci.*, 1942, **11**, No. 6.

Kow, T. H. .. "The Food and Feeding Relationships of the Fishes of Singapore Straits," *Colonial Office Fishery Publications*, 1950, **1**, No. 1, 1–35.

Ritchie, A. .. "The Food and Feeding Habits of the Haddock (*Gadus aeglefinus*) in Scottish Waters," *Fisheries, Scotland, Sci. Invest.*, 1937, No. II.

Sette, O. E. .. "Biology of the Atlantic Mackerel (*Scomber scombrus*) of North America—Part II. Migrations and Habits," *Bull. Fish and Wild Life Series, U.S. Dept. Interior*, 1950, **51**, 251–358.

Steven, G. A. .. "Contributions to the Biology of the Mackerel, *Scomber scombrus*" *Journ. Mar. Biol. Assoc.*. 1949, **28**, No. 3, 555–81.

Subrahmanyan, R. .. "A systematic Account of the Marine Plankton Diatoms of the Madras Coast," *Proc. Ind. Acad. Sci.*, 1946, **24**, No. 4, 85–197.

Venkataraman, R. and Chari, S. T. "Seasonal variation in the chemical composition of mackerel, *Rastrelliger kanagurta* (Russell)," *ibid.*, 1951, **33**, No. 3, 126–34.

DECOMPOSITION OF PADDY AND BAJRA (*PENNISETUM TYPHOIDEUM*) STRAWS BY FUNGI COMMONLY FOUND IN ALLAHABAD SOILS

By Ravi Prakash and R. K. Saksena
(*Botany Department, University of Allahabad*)

Received July 9, 1952
(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

Introduction

Importance of plant residues in soil fertility is now universally recognised. In the United States, plant residues are being used to improve the fertility of soil in many agricultural areas. In India, Pani (1951) used this technique in West Bengal for the same purpose. He ploughed the paddy straw left in fields from the earlier crop, and mixed it along with other fertilizers with the soil. The yield of paddy by this treatment was 73 maunds 30 seers per acre as compared to the Indian average of 12·5 maunds.

Before the residues are of any use to the plants, they have to be decomposed. This decomposition, as is well known, is influenced by climatic factors, and also by the micro-organisms present in the residues and the soil. Dawson (1949) studied the decomposition of wheat straw by some fungi commonly found in Nebraska soils. Norman (1929–31), Smith and Brown (1934), Phillips, Weihs and Smith (1930) and Waksman (1931) have shown disappearance of cellulose, hemicelluloses, lignin and pentosans during decomposition. Several species of *Aspergillus*, *Sepedonium*, *Trichoderma*, *Penicillium*, *Cladosporium*, *Mucor* and *Fusarium* were found decomposing various straws by Norman (1929–31), Thaysen and Baker (1927), Martin (1927), Tyner (1948) and Sadasivan (1939).

With a view to understand a more effective use of crop residues in increasing the fertility of the soil, decomposition of paddy and *bajra* straws by 22 fungi, previously isolated from Allahabad soils, was studied under controlled laboratory conditions.

Materials and Methods

The effect of the following 22 fungi isolated from Allahabad soils grown on Czapek's agar slant on the decomposition of paddy and *bajra* straws was studied:

UNIVERSITY OF MICHIGAN LIBRARIES
V 36 1952 XUM

Absidia heterospora, *Cunninghamella bertholletiæ*, *Cunninghamella echinulata*, *Syncephalastrum racemosum*, *Pythium* sp., *Pseudogymnoascus roseus*, *Chætomium* sp., *Aspergillus repens*, *Aspergillus proliferans*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus sydowi*, *Aspergillus niger*, *Aspergillus alliaceus*, *Aspergillus wentii*, *Aspergillus flavus*, *Aspergillus ochraceous*, *Penicillium luteum*, *Penicillium fellutanum*, *Pæcilomyces varioti*, *Curvularia lunata*, and *Neocosmospora vasinfecta*.

The methods employed were nearly the same as those of Dawson (1949) with some modifications as given below. The carbon dioxide produced during decomposition was absorbed in a standard solution of barium hydroxide kept in Petenkoffer's tube and titrated with N/8 hydrochloric acid solution. The carbon dioxide estimations were made daily for 7 days, while ammonia determinations were made at the end of the seventh day. All determinations were carried out in triplicate at room temperature. In the case of *bajra* straw total carbon decomposed was also estimated in order to determine the relation between the amount of total carbon decomposed and the loss in weight of the straw.

The total amount of carbon present in the straw, before and after decomposition, was determined by the Kjeldahl's method. 0·1 gram of the finely powdered straw was placed with about 0·2 gram of crystallised copper sulphate and about 5 grams of pure acid potassium sulphate in a long necked round-bottomed Jena flask of 300 c.c. capacity and 30 c.c. of pure concentrated sulphuric acid was added from a pipette. The flask was heated for about 4 hours, resulting in the evolution of sulphur dioxide which was absorbed in a standard solution of iodine. The total amount of carbon present in the straw was calculated by determining the amount of sulphur dioxide evolved.

The pH was determined by Kuhn's colorimetric method (Piper, 1944, p. 28).

The oxidase reaction was studied by growing the fungi on 0·25 and 0·5 per cent. tannic acid agar which was prepared as follows:—

(*a*)	Agar	20 grams
	Malt	20 grams
	Distilled water ..	500 c.c.
(*b*)	Tannic acid	2·5 grams or 5 grams
	Distilled water ..	500 c.c.

(*a*) and (*b*) were sterilised separately at 15 pounds pressure for 15 minutes and then mixed together aseptically before use.

A dark hallo around the colony indicated that the fungus is oxidase positive in reaction. Medium without tannic acid was used as control.

Results

The results of the decomposition of paddy and *bajra* straws, given in Tables I and II and Figs. 1 and 2 respectively, show that there is no uniformity in the rates of decomposition by various fungi. In some, *viz.*, *Absidia heterospora*, *Cunninghamella bertholletiæ*, *Cunninghamella echinulata*, *Syncephalastrum racemosum*, *Pythium* sp., *Pseudogymnoascus roseus*, *Aspergillus repens*, *Aspergillus proliferans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus alliaceus*, *Aspergillus ochraceous* and *Penicillium fellutanum*, the amount of carbon dioxide evolved was more than the loss in weight of the straw during decomposition, while in others, *viz.*, *Chætomium* sp., *Aspergillus fumigatus*, *Aspergillus sydowi*, *Aspergillus wentii*, *Aspergillus flavus*, *Penicillium luteum*, *Pæcilomyces varioti*, *Curvularia lunata* and *Neocosmospora vasinfecta*, the amount of carbon dioxide was less than the loss in weight. There were lot of variations in the amount of carbon dioxide given off in both the cases. Of the fungi tested, *Penicillium luteum* and *Pæcilomyces varioti* were the least active.

The cellulose equivalent of carbon dioxide evolved was not proportional to the loss in weight. Similar results have been reported by Dawson (1949, p. 474).

In order to determine relation between carbon dioxide evolution and loss in weight, the decomposition of the *bajra* straw and also the total loss of carbon during decomposition was studied. The data were analysed statistically.

Cellulose, being the chief constituent of the straw, undergoes the following chemical change during decomposition:—

$$(C_6H_{10}O_5)\,n + n6O_2 \rightarrow n6CO_2 + n5H_2O$$

It is evident that the straw is decomposed, resulting in the evolution of carbon dioxide and some loss in weight. Their correlation was studied statistically. The data were obtained from decomposition of paddy and *bajra* straws by 22 fungi and represent 44 observations. The results are shown graphically in Fig. 3 where loss in weight of the straws is plotted against the cellulose equivalent of the carbon dioxide evolved.

The equation of regression line in Fig. 3 is as follows:—

$$E = \hat{y} + \frac{Sxy}{Sx^2}(X - \hat{x}) = \cdot 47X + 266 \cdot 58$$

TABLE I

Name of the fungus	CO_2 evolved in mg.	Cellulose equivalent of CO_2 in mg.	Loss in weight in mg.	pH	Ammonia evolved in mg.	Oxidase reaction
Absidia heterospora ..	217·8	113·72	153·2	7·2	0·17	−
Cunninghamella bertholletiæ ..	446·1	273·90	419·3	7·4	0·08	−
Cunninghamella echinulata ..	262·8	161·36	135·5	7·0	0·08	−
Syncephalastrum racemosum ..	238·2	146·25	220·2	8·0	0·08	−
Pythium sp. ..	345·8	212·32	255·4	7·5	0·08	−
Pseudogymnoascus roseus ..	211·4	129·79	190·8	7·2	0·17	−
Chætomium sp. ..	307·7	188·92	623·2	8·0	0	−
Aspergillus repens ..	220·2	135·20	204·2	7·1	0·17	−
Aspergillus proliferans ..	246·1	151·10	176·0	7·6	0·08	−
Aspergillus fumigatus ..	415·3	255·09	512·4	7·4	0	−
Aspergillus nidulans ..	246·5	130·35	108·4	6·5	0·08	−
Aspergillus sydowi ..	262·2	160·99	315·5	6·3	0	−
Aspergillus niger ..	434·2	266·59	316·8	7·2	0·08	−
Aspergillus alliaceus ..	424·2	260·45	200·4	6·7	0·08	−
Aspergillus wentii ..	440·0	270·40	526·5	7·4	0·08	−
Aspergillus flavus ..	347·8	183·54	579·8	6·3	0	−
Aspergillus ochraceous ..	446·9	274·39	293·2	6·4	0·08	−
Penicillium luteum ..	154·8	95·04	629·8	6·3	0	+
Penicillium fellutanum ..	458·0	281·21	342·5	7·6	0·25	−
Pæcilomyces varioti ..	131·4	80·67	579·4	6·8	0·08	−
Curvularia lunata ..	457·3	280·78	516·2	7·3	0·08	+
Neocosmospora vasinfecta ..	191·8	117·76	373·4	6·5	0	−

TABLE II

Name of the fungus	CO_2 evolved in mg	Cellulose equivalent of CO_2 in mg.	Loss in weight in mg.	Total carbon decomposed in mg.	pH	Ammonia evolved in mg.	Oxidase reaction
Absidia heterospora ..	222·31	136·49	162·8	107·810	7·2	0·17	−
Cunninghamella bertholletiæ ..	403·59	247·80	402·2	404·685	7·4	0·08	−
Cunninghamella echinulata ..	222·31	136·48	120·6	104·685	7·0	0·17	−
Syncephalastrum racemosum ..	371·03	227·81	350·2	323·435	8·0	0·08	−
Pythium sp. ..	346·50	212·75	274·6	260·935	7·5	0	−
Pseudogymnoascus roseus ..	333·85	204·98	270·8	264·060	7·2	0·08	−
Chætomium sp. ..	414·37	254·32	650·8	579·685	8·0	0	−
Aspergillus repens ..	378·40	232·33	370·8	342·185	7·1	0·08	−
Aspergillus proliferans ..	267·34	164·14	197·4	192·185	7·6	0·08	−
Aspergillus fumigatus ..	452·43	267·79	500·2	498·435	7·4	0	−
Aspergillus nidulans ..	415·91	255·37	253·2	248·435	6·5	0·08	−
Aspergillus sydowi ..	378·07	232·13	514·4	498·435	6·3	0	−
Aspergillus niger ..	366·96	225·21	278·4	273·435	7·2	0·17	−
Aspergillus alliaceus ..	449·35	275·80	227·6	195·310	6·7	0	−
Aspergillus wentii ..	460·35	282·65	550·8	535·935	7·4	0·08	−
Aspergillus flavus ..	334·51	205·38	512·2	492·185	6·3	0·17	−
Aspergillus ochraceous ..	309·21	189·85	257·6	239·060	6·4	0	−
Penicillium luteum ..	274·55	168·57	660·0	576·560	6·3	0·08	+
Penicillium fellutanum ..	390·28	239·68	244·4	242·179	7·6	0·17	−
Pæcilomyces varioti ..	173·01	106·22	581·2	554·485	6·8	0·08	−
Curvularia lunata ..	429·99	264·01	510·2	485·935	7·3	0·08	+
Neocosmospora vasinfecta ..	168·52	103·47	310·6	320·333	6·5	0	−

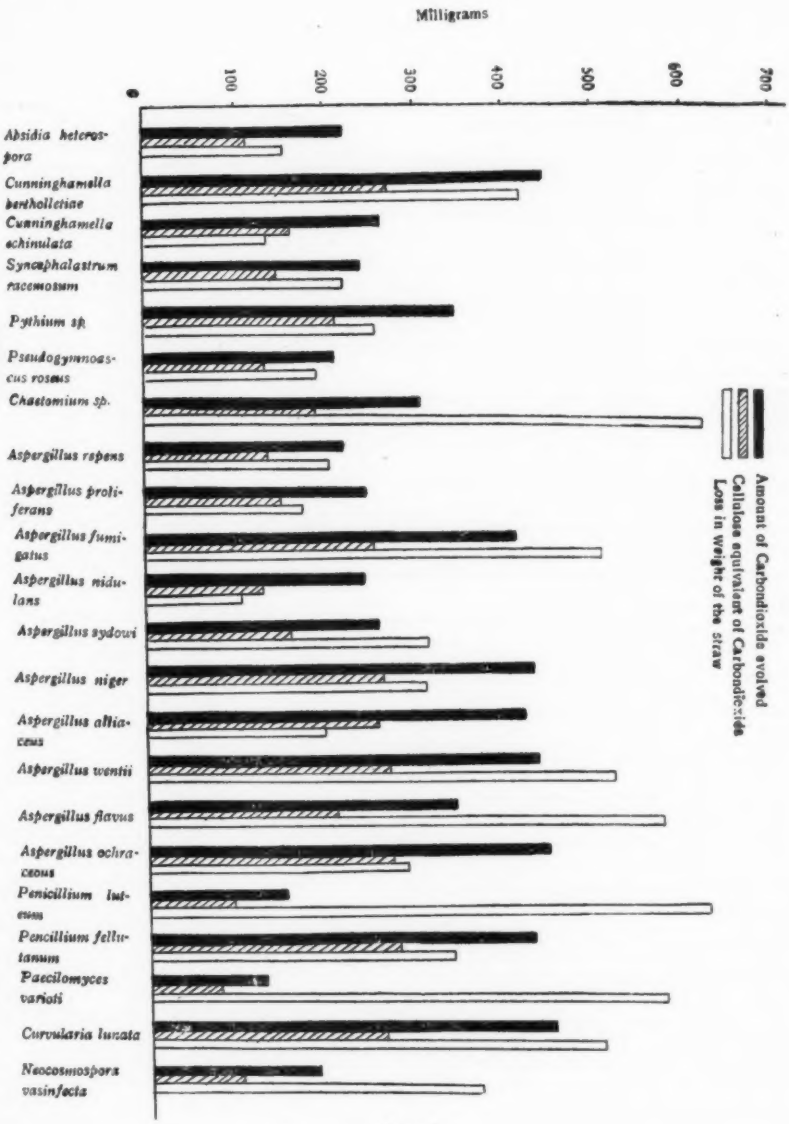


FIG. 1

The standard error $Sb = \frac{Syx}{\sqrt{Sx^2}} = \cdot 4099$ and the test significance $t = 1 \cdot 148$. The degree of freedom being 42, its value is below the value of t at 1% point.

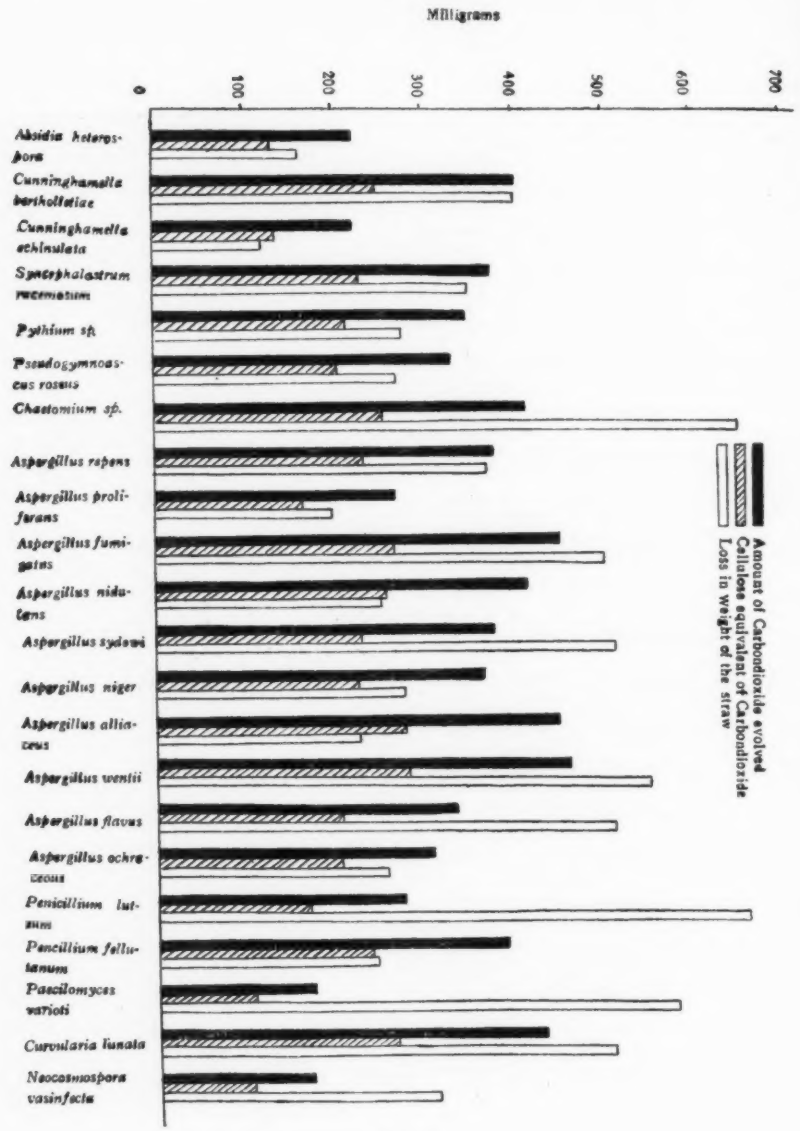


FIG. 2

The correlation coefficient $r = \frac{Sxy}{\sqrt{(Sx^2)\,(Sy^2)}} = \cdot 1769$, is also little less than the value of r at 1% or 5% point. Therefore, the correlation between the

cellulose equivalent of the carbon dioxide evolved and the loss in weight of the straws is not significant. The total amount of carbon dioxide evolved, therefore, does not account for the total loss in weight of the straws.

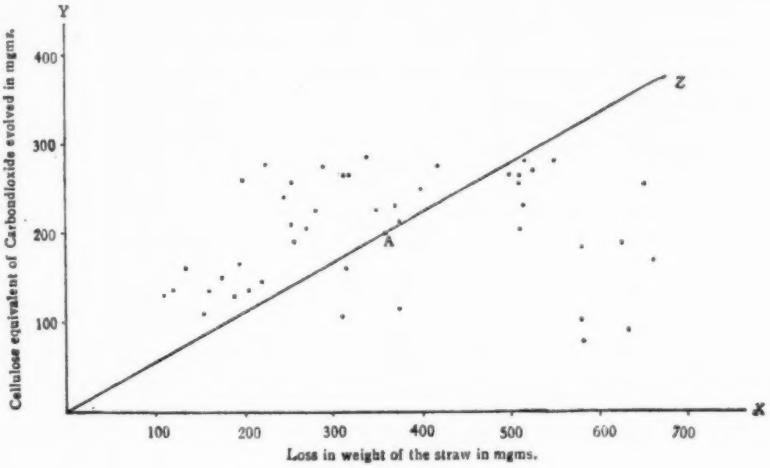


FIG. 3

In the case of the *bajra* straw the total loss of carbon during decomposition was studied and the data were analysed statistically. The results are shown graphically in Fig. 4 where the total loss of carbon is plotted against the loss in weight of the straw. The equation of regression line in Fig. 4 is as follows:

$$E = \bar{y} + \frac{Sxy}{Sx^2}(X - \bar{x}) = \cdot 743 + 1 \cdot 05X$$

The correlation coefficient $r = \cdot 9985$. The degree of freedom being 21, the value of r exceeds the value required at 1% point. The correlation, therefore, between the total loss of carbon and the loss in weight of the straw is significant. It is, therefore, clear that during decomposition carbon sources other than cellulose were used by fungi.

The maximum decomposition in the majority of cases was on the 6th day. In some, *viz.*, *Cunninghamella bertholletiæ*, *Aspergillus niger*, *Penicillium fellutanum* and *Curvularia lunata*, the maximum decomposition reached on 3rd day, in *Aspergillus fumigatus* and *Aspergillus wentii* on 4th day and in *Cunninghamella echinulata*, *Pythium* sp. and *Chætomium* sp., on the 5th day, after which there was a decline in the activity.

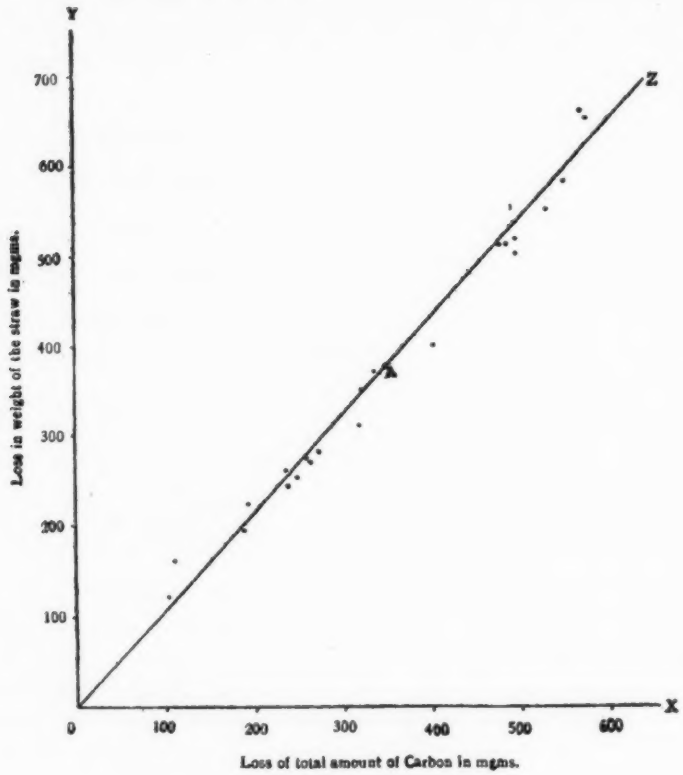


FIG. 4

There was a change in pH as a result of the decomposition of the paddy and *bajra* straws, the final pH value ranging from 6·3 to 8. *Syncephalastrum racemosum*, *Pythium* sp., *Chætomium* sp., *Aspergillus proliferans* and *Penicillium fellutanum* produced a definite alkaline reaction. A mild alkaline reaction was produced by *Absidia heterospora*, *Cunninghamella bertholletiæ*, *Pseudogymnoascus roseus*, *Aspergillus repens*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus wentii* and *Curvularia lunata*. *Cunninghamella echinulata* showed neutral reaction. The remaining fungi, *viz.*, *Aspergillus nidulans*, *Aspergillus sydowi*, *Aspergillus alliaceus*, *Aspergillus flavus*, *Aspergillus ochraceous*, *Penicillium luteum*, *Pœcilomyces varioti* and *Neocosmospora vasinfecta* showed an acidic reaction. There did not appear any correlation between the change in pH and the rate of decomposition as also reported by Dawson (1949).

When tested for their oxidase activity, only *Penicillium luteum* and *Curvularia lunata* proved to be oxidase positive, while the rest of fungi used were oxidase negative. Members of the same genus varied in their oxidase activity, *e.g.*, *Penicillium luteum* was positive while *Penicillium fellutanum* was negative. Similar results have been obtained by Dawson (1949) in some undetermined species of *Penicillium*. There did not appear any correlation between the oxidase activity and the straw decomposition in the present tests. Dawson (1949) on the other hand, showed that oxidase negative fungi are less active than the oxidase positive and species of *Aspergillus*, which are oxidase negative, occupy an intermediary position.

The amount of ammonia evolved during decomposition was very little (Tables I and II).

All the 22 fungi used showed almost similar behaviour towards both paddy and *bajra* straws (Tables I and II).

The results given in Table II show that in each case there is much correlation between the amount of total carbon decomposed and the total loss in weight of the straw.

Summary

The aerobic decomposition of paddy and *bajra* straws by 22 fungi isolated from Allahabad soils was studied under controlled laboratory conditions. Dawson's methods were employed with certain modifications. The decomposition was studied by determining the carbon dioxide and ammonia evolution, loss in weight and loss in total amount of carbon during a 7-day period.

The fungi were tested for their oxidase activity and out of 22 fungi only two were found to be oxidase positive.

There is no uniformity in the rates of decomposition by various fungi. In certain cases the amount of carbon dioxide evolved was more than the loss in weight and in other cases the amount of carbon dioxide evolved was less than the loss in weight of the straw. The cellulose equivalent of carbon dioxide evolved was not proportional to the loss in weight.

As a result of decomposition the change in pH of the straw was in some cases towards the acidic side and in other towards the alkaline. Further, the rate of decomposition has no relation either with change in pH of the straw or with the oxidase activity of the fungi.

There was a fair amount of correlation between the total carbon decomposed and the total loss in weight of the straw.

All the 22 fungi behaved similarly towards paddy and *bajra* straws.

ACKNOWLEDGEMENTS

The authors wish to make grateful acknowledgements to the Uttar Pradesh Government Scientific Research Committee for the award of a Research Assistantship (to Shri Ravi Prakash) and research contingencies. The authors are grateful to Shri D. N. Elhance of the Commerce Department of the University of Allahabad for going through the statistical portion of the paper.

REFERENCES

Clarke, H. T. .. *A Hand Book of Organic Analysis*, pp. 303–06.

Dawson, R. C. .. "Effect of crop residues on soil micro-populations, aggregation and fertility under Maryland conditions," *Soil Sci. Soc. Amer. Proc.* (1945), 1946, **10**, 180–84.

——— .. "Decomposition of wheat straw by some fungi commonly found in Nebraska soils," *Soil. Sci.*, 1949, **67**, 467–79.

Doryland, C. J. T. .. "The influence of energy material upon the relation of soil micro-organisms to soluble plant food," *N. Dak. Agr. Exp. Sta. Bul.*, 1916, 116.

Duley, F. L. and Russel, J. C. "The use of crop residues for soil and moisture conservation," *Jour. Amer. Soc. Agron.*, 1939, **31**, 703–09.

Ledingham, G. A. and Adams, G. A. "Biological decomposition of chemical lignin, II," *Canad. Jour. Res.* (C), 1942, **20**, 13–27.

Martin, T. L. .. "Decomposition studies of alfalfa and sweetclover roots and straw," *Soil Sci.*, 1927, **24**, 309–16.

Murray, T. J. .. "The effect of straw on biological soil processes," *ibid.*, 1921, **12**, 233–59.

Norman, A. G. .. "The biological decomposition of plant materials, II," *Bio. Chem. Jour.*, 1929, **23**, 1367–84.

——— .. "The biological decomposition of plant materials, III," *Ann. Appl. Biol.*, 1930, **17**, 575–613.

——— .. "The biological decomposition of plant materials, IV," *ibid.*, 1931, **18**, 244–59.

Pani, J. C. .. "West Bengal Farmer's Record Paddy Yield," *A. B. Patrika*, Calcutta, February 26, 1951, **83**, No. 57, p. 8, Col. 3.

Phillips, M., Weihe, H. D. and Smith, N. R. "The decomposition of lignified materials by soil micro-organisms," *Soil Sci.*, 1930, **30**, 383–90.

Piper, C. S. .. *Soil and Plant Analysis*, University of Adelaide, 1944.

Sadasivan, T. S. .. "Succession of fungi decomposing wheat straw in different soils with special reference to *Fusarium culmorum*," *Ann. Appl. Biol.*, 1939, **26**, 497.

Smith, F. B. and Brown, P. E. "The decomposition of straw in the production of artificial manure," *Iowa State Col. Jour. Sci.*, 1934, **8**, 409–13.

Snedecor, G. W. .. "Statistical Methods," Iowa State College Press, *Ames. Iowa*, 1940.

Thaysen, A. C. and Bakes, W. E. "On the early stages of microbial decay and humification of vegetable tissues," *Bio-chem. Jour.*, 1927, **21**, 895–900.

Tyner, L. E. .. "Effect of crop debris, plant roots and crop sequence on the microbial flora of the soil in relation to root rot cereal crops," *Canad. Jour. Res.* (C), 1948, **26**, 86–93.

Waksman, S. A. .. "Decomposition of the various chemical constituents of complex plant materials by pure cultures of fungi and bacteria," *Arch. Microbiol.*, 1931, **2**, 136–54.

GLOMERELLA PSIDII (DEL.) SHELD. AND *PESTALOTIA PSIDII* PAT. ASSOCIATED WITH A CANKEROUS DISEASE OF GUAVA

By N. S. Venkatakrishniah

(*Assistant Plant Pathologist, Mysore Agricultural Department, Bangalore*)

Received March 25, 1952

(Communicated by Prof. L. Narayana Rao, F.A.SC.)

A CANKER or scab of guava fruits (*Psidium guajava* L.) occurs seriously in Mysore on several local and grafted varieties. *Colletotrichum* and *Pestalotia* are observed on the affected fruits and leaves. *Colletotrichum* and *Glœosporium* on guava fruits have been mentioned by Narasimhan (1931–40) in Mysore. *Colletotrichum psidii* according to Dey (1934) occurs in the United Provinces and according to Mehta (1951) in Uttar Pradesh. *Pestalotia psidii* on guava has been reported by Patel, Kamat and Hingorani (1950) at Poona. Curzi (1927) describes *Colletotrichum psidii* on leaves of guava in Italy. Ocfemia (1931) reports that *Glomerella psidii* in the Philippines is very similar to *Glomerella cingulata* (Stonem.) S. and v. S. According to Shear and Wood (1907), Sheldon obtained the ascogenous stage of the guava anthracnose *Glomerella psidii* (Del.) Sheldon in pure cultures. There appears to be no record of *Glomerella psidii* in India. Since various organisms are associated with the canker of guava, it was thought desirable to investigate the exact cause of the disease.

Symptoms.—The characteristic symptom of the disease is the development of canker on young green and mature fruits. Small circular spots appear on buds, calyx and leaves. The spots enlarge, turn black or brown and unite with one another. In severe cases raised, cankerous spots develop in great numbers and the fruit breaks open to expose the seeds (Fig. 1). The infected fruits remain undeveloped, become hard, malformed and mummified. They drop in great numbers. Sometimes small, rusty brown, angular spots appear on the leaves (Fig. 2).

Isolation.—Repeated attempts to isolate the fungus from infected young buds and calyx were of no avail. The two fungi were isolated in November 1949 from leaves and fruits. *Colletotrichum* and *Pestalotia* both appeared sometimes from the same bit, one overgrowing the other and sometimes individually.

Colletotrichum psidii grew well on oat and potato dextrose agars, but thin on maltin agar. The acervuli were black, round and gregarious. Setæ were not constant. The conidia were salmon-coloured in mass but hyaline singly, unicellular, granular, vacuolate, rounded at both ends, sometimes slightly curved, and measured 10–24×3·5–7 μ. They germinated readily in water, more so in tap than in distilled water. The germ tube put forth characteristic appressoria. Perithecia developed on oat and maltin agars at laboratory temperature in four weeks. They were dark coloured, globular, aparaphysate and measured 112–192×176–288 μ on oat agar. The asci (Fig. 5) were numerous, hyaline, sessile, thin walled, clavate or subcylindrical and measured 31·5–68×8–13·5 μ. The ascospores (Fig. 6) were eight in number, elliptical, sometimes slightly curved, hyaline, unicellular, granular and measured 13·5–20·5×4–8 μ. The perithecial stage of certain species of *Glomerella* has been observed only on host tissues and of certain other species only in artificial cultures. The perfect stage of the fungus on guava has not been observed in Nature. That obtained in culture is similar to *Glomerella psidii* reported by Ocfemia (1931) and Shear and Wood (1907).

Pestalotia psidii grew well on oat agar. The mycelium made a thick, pure white, cottony growth and acervuli developed as black, shining, moist crusts in cultures. The conidia were spindle shaped, dark coloured in mass and 5-celled. The upper end conical cell bears usually 3 long, slender, colourless, simple appendages. 2–5 are sometimes met with. The appendages measure 7–17 μ long and the conidia 17–24×5–7 μ on the host. The conidia germinated readily in sterile distilled water in 3 hours, usually from the hyaline end cells.

Inoculation Experiments.—Inoculations with the two fungi from pure cultures were conducted under aseptic conditions on fresh, young, green guava fruits and leaves of several grafted " Banaras ", " Bangalore " (pear-shaped), " Allahabad ", " Safeda ", and grafted (seedless) varieties obtained from Hessarghatta Fruit Research Station. The twigs bearing the fruits and leaves were left standing in water or in 2·5% sugar solution and covered with big bell jars. 40–60 fruits of about the same size borne on a grafted tree were inoculated under aseptic conditions with the two fungi. The inoculated fruits and leaves on the tree were covered with moist cotton wads and wrapped loosely with waxed paper bags. Conidia of *Colletotrichum* and *Pestalotia* from single conidium cultures were suspended separately in sterile distilled water. The two suspensions were placed individually and in combination within marked regions on the upper and lower surfaces of leaves and on the fruits. Some were inoculated by placing the two suspensions very close together on the same fruit. The inoculated regions of some fruits

were injured by pinpricks and kept moist by atomizing water for 4-6 days. Both the fungi infected readily the fruits and leaves of all the varieties except " Allahabad ", " Safeda " and grafted (seedless) fruits.

The regions of fruits inoculated with *Colletotrichum* developed a water soaked appearance and the spots extended in diameter in 8–10 days. The tissue beneath turned brownish in colour. Leaves inoculated on the lower surface took a pinkish colour at first and then turned brownish in 6–8 days. Conidia of *Colletotrichum* were observed in abundance. Sections of the tissue showed the mycelium. The fungus was reisolated and resembled the original isolation.

There was a fluffy growth of the mycelium at the regions inoculated with *Pestalotia*. Sections of the infected regions showed the mycelium in the tissue. The leaves inoculated on the lower surface took the infection 2–3 days earlier than those inoculated on the upper surface. The infected regions turned dark brown and varied in size from 2 to 10 mm. in 8 days (Fig. 4). Conidia of *Pestalotia* were observed on the infected spots, more predominantly on the fruits. The inoculated fruits turned black and remained hard for over 20 days. This symptom corresponded to that observed in Nature. The fungus from the inoculated leaves and fruits was reisolated and was found to resemble the original isolation.

Either *Pestalotia* or *Colletotrichum* predominated when both the fungi were inoculated together on the same fruit, one fungus soon outgrowing the other. The controls remained healthy.

Inoculation experiments were conducted in the laboratory under aseptic conditions with the two fungi on young fruits of apple (*Pyrus malus* L.), brinjal (*Solanum melongena* L.), chilly (*Capsicum annuum* L.), French bean (*Phaseolus vulgaris* L.), lemon (*Citrus aurantifolia* Swingle), mango (*Mangifera indica* L.), papaya (*Carica papaya* L.), plantain (*Musa sapientum* L.) and tomato (*Lycopersicum esculentum* Mill.). The infection by *Pestalotia* was not observed on any of the fruits. Infection by *Colletotrichum* was observed on apple, chilly, lemon, mango, papaya, plantain and tomato, but not on brinjal and French bean. It was characteristic on apple fruits. The infected circular spot extended gradually with a dark brown discolouration. It was more rapid on tender yellowish white fruits of papaya than on small green fruits. Conidia of *Colletotrichum* were observed in abundance at the inoculated regions. The controls were healthy. This confirms the work of Ocfemia (1931) in Philippines who succeeded in infecting chilly, egg plant, tomato, avocado, banana and mango with *Glomerella psidii*.

Patel, Kamat and Hingorani (1950) were not successful in artificially infecting the leaves of guava with *Pestalotia psidii* Pat. But the author's experiments conducted with both the fungi showed infection readily on the injured fruits and leaves (Figs. 3 and 4) in 4 or 5 days except on the fruits of " Allahabad ", " Safeda " and grafted (seedless). The virulence varied with the varieties. The incidence of infection was 66 per cent. with *Pestalotia* and 57 per cent. with *Colletotrichum*.

CONCLUSION

Colletotrichum psidii Curzi and *Pestalotia psidii* Pat. appear to be weak parasites infecting readily the fruits and leaves of guava injured by pinpricks. In Nature the insect *Helopeltis* which is commonly met with in gardens probably causes the initial injury on fruits and leaves. The infection by fungi follows later on. I am thankful to Dr. M. Puttarudriah, Senior Assistant Entomologist of this Department, for the information that his experiments with the species *Helopeltis antonii* Signoret produced the disease symptoms on guava fruits and leaves in Mysore. Hopkins (1950) states that leaf spot, and fruit scab due to *Pestalotia psidii* follows insect punctures by *Helopeltis sanguineus*. Leach (1935) has shown that a stem canker, an angular spot a fruit scab and a fruit rot of mango are caused by the bug *Helopeltis bergrothi* Reut. and that the symptoms are similar to those caused by many fungi and bacteria.

Control.—Narasimhan (1938–40–41) and Venkatarayan (1937) report the experiments done to control the disease. The spraying experiments with 1% Bordeaux mixture and lime sulphur solution diluted 1 : 25 were conducted on a block of 25 plants in a garden near Bangalore in 1949–50. All the cankered fruits and leaves, mummies, dried and dropped fruits were collected and destroyed before the treatment. Three to four sprayings at intervals of 15 days were given when the fruits began to set. The infection which was confined to a few plants in a corner of the garden at the time of the first spraying did not spread to other sprayed trees. The sprayed plants produced good sized fruits.

SUMMARY

The cankerous disease of guava (*Psidium guajava* L.) exhibiting characteristic spots and malformations is prevalent in Mysore. It is associated with two fungi *Colletotrichum psidii* Curzi and *Pestalotia psidii* Pat.

Colletotrichum psidii developed a perithecial stage in pure culture for the first time in India. The perithecia are globular, dark coloured, aparaphysate. Asci are numerous, hyaline and the ascospores are 8 in number, unicellular and granular. This stage corresponds closely to *Glomerella psidii* (Del.) Sheld.

Colletotrichum psidii infected the fruits of apple, chilly, lemon, papaya, plantain, mango and tomato and not brinjal and French bean. *Pestalotia psidii* did not infect any of these hosts. Both the fungi infected the injured fruits and leaves of guava.

Colletotrichum psidii is a general parasite while *Pestalotia psidii* is specialised to guava.

Three or four sprayings with 1% Bordeaux mixture or with lime sulphur solution 1 : 25 done at intervals of 15 days at an early stage of infection controls the spread of the fungi.

The author is thankful to Sri. S. V. Venkatarayan, Plant Pathologist, for his interest and helpful criticism in the course of this work.

Literature Cited

Curzi, M. .. "De novis Eumycetibus. (Concerning new Eumycetes.)," *Atti Ist. Bot. R. Univ. di Pavia*, 1927, **3**, 203–08, Abst. *Rev. App. Mycol.*, 1928, **7**, 744.

Dey, P. K. .. "A disease of guava fruits in the United Provinces," *Int. Bull. Plant Protection*, 1934, **8**, 30 M.

Hopkins, J. C. F. .. "A descriptive list of plant diseases in Southern Rhodesia and list of bacteria and fungi," *Mem. Dept. Agric.*, 1950, No. 2, 57.

Leach, R. .. "Insect injury simulating fungal attack on plants. A stem canker, an angular spot, a fruit scab and a fruit rot of mangoes caused by *Helopeltis bergrothi* Reut. (Capsidae)," *Ann. Appl. Biol.*, 1935, **22**, 525–37.

Mehta, P. R. .. "Observations on new and known diseases of crop plants of the Uttar Pradesh," *Plant Protection Bull.*, 1951, **3**, **1**, 11–12 (Ministry of Food & Agric., Govt. of India).

Narasimhan, M. J. .. "Report of work done in the Mycological Section" In *Ann. Repts. Mys. Agric. Dept.*, 1929–30, 1931, 24; 1936–37, 1938, 172 ; 1938–39, 1940, 96–97; 1939–40, 1941, 255.

Ocfemia, G. O. .. "Notes on some economic plant diseases new in the Philippine Islands. II." *Phlipp. Agric.*, 1931, 19.

Patel, M. K., M. N. Kamat, and G. M. Hingorani "*Pestalotia psidii* Pat. on guava," *Indian Phytopathology*, 1950, III, **2**, 165–76.

Shear, C. L., and Anna K. Wood "Ascogenous forms of *Glœosporium* and *Colletotrichum*," *Bot. Gaz.*, 1907, **43**, 259–66.

Venkatarayan, S. V. .. "Report of work done in the Mycological Section," *In Admn. Rept. Mys. Agric. Dept.*, 1935–36, 1937, 53.

EXPLANATION OF FIGURES

FIG. 1. Naturally infected guava fruits showing the cankerous spots and splitting.

FIG. 2. Naturally infected guava leaves showing numerous spots.

FIG. 3. Guava leaves inoculated with

A. *Pestalotia psidii* and
B. *Colletotrichum psidii* both showing the infection.

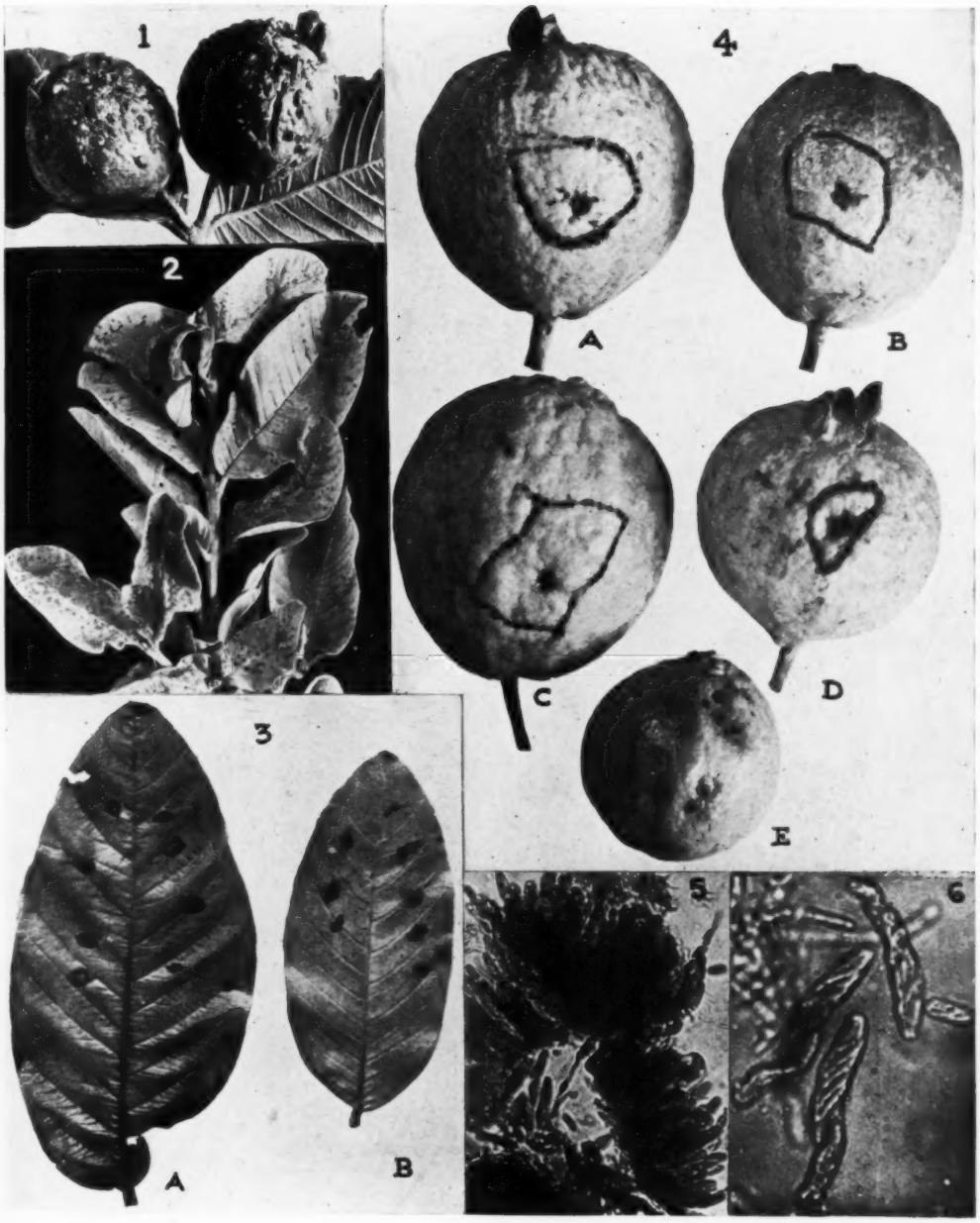
FIG. 4. Fruits of a grafted variety (Bangalore) showing the characteristic infection within marked regions after inoculation with :—

A. *Pestalotia psidii* isolated from fruit and
B. that of leaf.
C. *Colletotrichum psidii* from fruit.
D. *Colletotrichum psidii* from leaf and
E. a naturally infected fruit.

FIG. 5. Microphotograph of a perithecium and extruded asci, × 280.

FIG. 6. Microphotograph of three asci showing ascospores, × 680.

710-52 Printed at the Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore.


1
2
3
A
B
4
A
B
C
D
E
5
6

SPORES AND POLLEN FROM TERTIARY LIGNITES FROM DANDOT, WEST PUNJAB (PAKISTAN)

By K. P. Vimal, M.Sc.

(Geology Department, Lucknow University, Lucknow)

Received June 26, 1952

[Communicated by Prof. L. Narayana Rao, M.Sc. Ph.D. (London), F.L.S., F.A.SC.]

Introduction

The material, described in this paper, was very kindly handed over to me by Dr. R. C. Misra (Reader in Geology Department of Lucknow University), who collected it from one of the two coal seams found at Dandot in the village of Dandot, in Jhelum District, Punjab (W. Pakistan). The coal seams occur a foot below the nummulitic limestone and are generally regarded as Eocene in age (La Touch, 1897). The friable and jointed coal is lignitic like that of Palana but is more compact, bright and blacker.

The macerations were done inside the fume chamber and all precautions were taken to prevent laboratory contamination. The material was directly heated in a flame before transferring it to the macerating fluid, and the maceration tubes were kept under bell jars. The material was then kept for ten days in Schulze's solution and then repeatedly washed. It was next treated with 10% sol. of potassium hydroxide for about three hours. Finally it was washed and centrifuged. The permanent mounts were made with glycerine jelly. Only those forms which repeatedly occur in different slides prepared from different chips of materials, have been taken into account. Wherever possible both the equatorial and polar views of the pollen grains have been studied, sketched and photographed. Often the grains are so firmly fixed that they cannot be moved, even after warming the slide. In such cases I could sketch or photograph the grain in a particular view only. To facilitate comparisons, all the types have been drawn and photographed at the same magnifications.

Terminology

The terms used in the detailed structural descriptions of the types in this paper are borrowed from Wodehouse (1935), Erdtman (1943, 1947) and Selling (1946, 1947).

NOMENCLATURE

Previous workers like Potonie, R. (1931), Kirchheimer (1931–32) and other earlier investigators have employed the term *Pollenites* or *Pollinites* for fossil pollen and *sporites* for fossil spores. They have further adopted a binomial nomenclature for the pollen. R. A. Couper and others further designate the fossil pollen according to the modern genera and species which they resemble. The comparison of fossil pollen with the living ones cannot be perfect. Firstly we do not know the pollen characters of all living plants. Secondly, mode of preservation, variation in size and other factors render it difficult to make correct comparisons. In view of these difficulties I have not attempted to refer the types in this paper to the living genera and species as suggested by Couper and others. On the other hand, I have put my pollen and spores under the term '*Sporomorph*' and have arranged them according to the noncommittal classification of Erdtman (1947).

CLASSIFICATION

Various systems of classification of fossil pollen and spores have been put forth by different workers: Fisher (1890), Potonie, H. (1893), Ibrahim (1933), Raistric and Simpson (1933), Wodehouse (1933), Naumova (1937), Selling (1946–47), Erdtman (1947), Faegri and Iverson (1950) and Hofmann (1950). But out of these, the classifications of Wodehouse (1933), Ibrahim (1933), Naumova (1937) and Erdtman (1947) are preferable because they take into consideration a large number of morphological features, like furrows, pores and exine characters, etc. Erdtman's classification is based upon a study of the maximum morphological features of the living pollen. I have therefore adopted this classification.

COMPARISONS

Comparison of fossil pollen and spores with living pollen and spores is a very important but at the same time very difficult task. Fossil pollen and spores are likely to have suffered in size, shape and surface features during fossilization due to various factors. There is, with living pollen and spores, a range of variation in size, shape, etc., which are dependent upon various ecological factors, like temperature, climate, nutrition (Schose Bodmer, 1940), etc. In fact we do not have complete knowledge, description, and figures of living pollen and spores to make any kind of definite comparisons between them and fossil types. I have therefore refrained from making any definite comparisons. But wherever resemblances between the fossil and living types have been observed, attention has been drawn to them.

Descriptions

Trilites (Erdtman, 1947)

Trilites spm. 1.—Spore tetrahedral, brown. Oblique proximal view (Pl. V, Fig. 1; Pl. VII, Fig. 1) subtriangular ($41 \cdot 5\,\mu \times 40 \cdot 3\,\mu$). Trilite mark short. Exospore dark brown in colour, surface sculpture scrobiculate. The spore can be put under the type *Triletes* and subtype *punctati-sporites* of Ibrahim (1933). No perine or perisporium is visible. Without making any definite comparison it might be pointed out that the spore resembles in appearance, size and surface sculpture, the spores of *Ophioglossum falcatum* (Sellimg, 1946, p. 26, Figs. 31–32). Slide No. S.R. 12.

Trilites spm. 2.—Spore tetrahedral, brown. Proximal view (Pl. V, Fig. 2; Pl. VII, Fig. 2), triangular with rounded angles and straight sides, ($42 \cdot 8\,\mu \times 41 \cdot 6\,\mu$). Distal side slightly convex. Exospore thick, brown, reticulate; meshes clearly formed and about 5–8 μ thick. Reticulum less pronounced round the apical crest. Tritete marks fairly long. It has not been possible to get the distal view. According to the classification put forth by Ibrahim (1933) the spore can be put under the type *Triletes* and subtype (*Reticulati-sporites*). No perine is visible. The spore resembles in shape, size and surface sculpture, the spores of *Lycopodium alpinum* (Erdtman, 1943, p. 148, Figs. 459–60) and *Ophioglossum vulgatum* (E. M. Knox, 1951, p. 438, Pl. Fig. 2) also. Slide No. S.R. 16.

Trilites spm. 3.—Spore large, tetrahedral, yellow. Proximal view (Pl. V, Fig. 3; Pl. VII, Fig. 3) triangular with slightly convex sides and well-rounded angles, $59 \cdot 5\,\mu \times 55 \cdot 4\,\mu$. Proximal side almost flat. Distal side convex. Triradiate mark extends upto the margin of the exospore which is $4 \cdot 7\,\mu$ thick. Surface sculpture scrobiculate, pits small. The spore can be put under the type *Triletes*, subtype *punctati-sporites* of Ibrahim. The spore resembles in shape and size the spore of *Schizostege Lydgatei*, but differs in surface sculpture (Selling, 1946, p. 60, Figs. 111–13). Slide No. S.R. 5.

Trilites spm. 4.—Spore large, tetrahedral, brown (Pl. V, Figs. 4–6; Pl. VII, Figs. 4–6). Pl. V, Fig. 4; Pl. VII, Fig. 4 show the equatorial view ($72 \cdot 2\,\mu \times 76 \cdot 1\,\mu$) of the spore. The distal side (*d*) is dome-shaped, the proximal side (*p*) is very slightly convex. There is a clear rim (*r*) where the two sides join. The triradiate mark extends from the pole to this rim. Exospore is $3 \cdot 5\,\mu$ thick brown in colour and has a smooth surface. Pl. V, Fig. 5; Pl. VII, Fig. 5 show the proximal part of a different but similar spore and the spore measures $78 \cdot 4\,\mu \times 63 \cdot 1\,\mu$. Pl. V, Fig. 6; Pl. VII, Fig. 6

are oblique views of another bigger spore (83·3 μ ×81·0 μ) probably of the same type. Slide No. S.R. 18.

Trilites spm. 5.—Spore large, tetrahedral, and brown (Pl. V, Figs. 7–8; Pl. VII, Figs. 7–8). Pl. V, Fig. 7; Pl. VII, Fig. 7 show the proximal view of the spore, which is triangular and measures 65·3 μ ×77·2 μ. The triradiate mark (*t*) seems to broaden out and the slit (*s*) also divides slightly a little away from the margin. Exospore 3·5 μ thick, smooth. Pl. V, Fig. 8; Pl. VII, Fig. 8 show another similar but slightly larger (91·7 μ ×74·9 μ) spore. Slide No. S.R. 12.

Trilites spm. 6.—Spore light yellow in colour, tetrahedral; proximal view (Pl. V, Figs. 9–10; Pl. VII, Figs. 9–10) triangular with broadly rounded angles, 98·7 μ ×102·2 μ. Triradiate mark very clear and each mark has on its two sides a thick area (*a* in Pl. V, Fig. 9). Each scar dilates into a thick rounded area, dark in colour. Exospore 2·5 μ thick and smooth. This is not found in another similar and smaller (94·0 μ ×88·2 μ) spore (Pl. V, Fig. 10; Pl. VII, Fig. 10). The exospore in Pl. V, Fig. 10, is slightly thicker opposite the ends of the tetrad scar. In spite of these little differences other features are so similar that there is little doubt that the two spores belong to the same form. This type resembles the *Trilites spm.* 5 in general form. But the size of the spore, the thickness of the exospore, the tetrad scar, its length and the nature of its termination, etc., are very different. So they are placed separately. Slide No. S.R. 12.

Trilites spm. 7.—Pl. V, Fig. 11; Pl. VII, Fig. 11 show the proximal view of another similar but smaller (64·1 μ ×62·9 μ) spore. The thickness of the exospore is 2·2 μ. In view of the similar form and surface features it is quite likely that this is only a young spore of the above discussed type. Slide No. S.R. 4.

Trilites spm. 8.—Spore comparatively small, yellow; proximal view (Pl. V, Fig. 12; Pl. VIII, Fig. 12) triangular with angles broadly rounded, 41·6 μ ×50·0 μ. Triradiate marks clear, short and reach half the distance between the centre and the margin. Exospore 1·5 μ thick and smooth. Slide No. S.R. 6.

Trilites spm. 9.—Spore small, yellow, tetrahedral. Pl. V, Fig. 13; Pl. VIII, Fig. 13 show the triangular proximal view 35·7 μ ×35·7 μ with the triradiate mark reaching right upto the margin. The angles are broadly rounded and the sides are slightly concave. This may be due to the flaccid condition of the spore, as is shown by the folds seen as lines on the spore in Pl. VIII, Fig. 13. Exospore smooth, 1·1 μ thick. This spore can be

compared with the spores of *Doryopteris decora* (Selling, 1946, p. 54, Pl. VI, Figs. 130–32). Slide No. S.R. 3.

Trilites spm. 10.—Pl. V, Fig. 14; Pl. VIII, Fig. 14 show the three spores of a single tetrad $29{\cdot}7\,\mu \times 29{\cdot}7\,\mu$ attached together. Pl. V, Fig. 15 is the same tetrad drawn at another focus to show the fourth spore, the other three are seen hazily. No detached spores of this type are seen. But judging from the tetrad itself the spore was small, yellow, and tetrahedral, $26{\cdot}1\,\mu \times 14{\cdot}3\,\mu$, with a convex distal side and a $1{\cdot}5\,\mu$ thick and slightly echinate exospore. The spore can be compared in shape and to a certain extent in the echinate nature of the exospore with the spores of *Selaginella deflexa* (Selling, 1946, p. 21, Figs. 24–25, Pl. I). But the difference lies in that my specimen shows shorter echinæ and is smaller in size while those figured by Prof. Selling are bigger and have longer echinæ. Slide No. S.R. 3 and S.R. 2.

Pollen Grains

Nonaperturites (Erdtman, 1947)

Subpilonapites spm. 1.—Grain large, spherical, brown, showing probably polar view (Pl. VI, Fig. 16; Pl. VIII, Fig. 15), $38{\cdot}1\,\mu \times 41{\cdot}6\,\mu$. Pore or furrow absent. Exine thick, and uneven, surface covered by thick warts densely placed and giving a verrucose appearance to the surface. This grain resembles in surface sculpture and general form *Type No. 8* described from Palana, and referred to *Potomogeton natans* (Rao and Vimal, 1950, p. 84) but differs from it, in being a little bigger. Slide No. S.R. 2.

Reticulonapites spm. 1. Grain large, $43{\cdot}0\,\mu \times 38{\cdot}1\,\mu$, brown, oblong, flat, probably equatorial view (Pl. VI, Fig. 17; Pl. VIII, Fig. 16); exine $3{\cdot}5\,\mu$ thick and smooth, but the surface sculpture is reticulate. Slide No. S.R. 5.

Monocolpites (Erdtman, 1947)

Monocolpites spm. 1.—Pl. VI, Fig. 18; Pl. VIII, Fig. 17 show a brown, oval, discoid monocolpate grain which measures $45{\cdot}3\,\mu \times 59{\cdot}5\,\mu$. Furrow long, narrow $45{\cdot}3\,\mu \times 2{\cdot}3\,\mu$, does not touch the poles. Exine $3{\cdot}5\,\mu$ thick, smooth. The grain could not be sketched in any other view. Slide No. S.R. 5.

This grain could be put under *Monosulcites* (Erdtman, 1947), if it could be proved that the furrow is on the distal side. It has not been possible to make out this feature clearly, and so the grain has been classed as *Monocolpites* (Erdtman, 1947).

Monocolpites spm. 2.—Pl. VI, Fig. 19; Pl. VIII, Fig. 18 show probably the equatorial view of the grain which measures $38 \cdot 1 \mu \times 45 \cdot 3 \mu$. A long furrow $1 \cdot 5 \mu$ broad extends from pole to pole. Exine $3 \cdot 5 \mu$ thick with scrobiculate surface sculpture. Slide No. S.R. 13.

Since these grains occur as isolated specimens it is difficult to make out their distal and proximal sides and also the poles. It is quite likely that the furrow in (Pl. VI, Fig. 19 and Pl. VIII, Fig. 18) does not actually run from pole to pole but extends along the equator of the grain. It is also quite likely that the furrow present in the grain is on the distal side, then the grain will have to be placed under *Monosulcites* of Erdtman (1947).

Dicolpites (Erdtman, 1947)

Dicolpites spm. 1.—Grain small, flat and light yellow (Pl. VI, Fig. 20; Pl. VIII, Fig. 19) show perhaps the equatorial view which is circular in outline and measures $38 \cdot 1 \mu \times 36 \cdot 9 \mu$. Part of the flaccid grain is slightly folded up on the right side. Two small slit-like horizontal furrows are present, one on each side. Fig. 21 is the sketch of the same grain drawn at a different focus to show the furrow on the other side. Each furrow is $14 \cdot 3 \mu$ long and $1 \cdot 5 \mu$ broad. Exine $1 \cdot 5 \mu$ thick, smooth and hyaline. The surface of the grain looks very finely striated due to striæ (seen imperfectly in the photo) on the ektexine. Slide No. S.R. 11.

Tricolpites (Erdtman, 1947)

Tricolpites spm. 1.—Grains small, brown, spheroidal, but slightly compressed due to pressure during preservation (Pl. VI, Fig. 22; and Pl. VIII, Fig. 20) show the oblique polar view, $27 \cdot 3 \mu \times 28 \cdot 5 \mu$. Ektexine thin, hyaline, $1 \cdot 1 \mu$. Intexine $1 \cdot 0 \mu$ thin, brownish. Surface of the grain faintly granular. Colpæ thin and long but do not extend to the poles. Each flange is $20 \cdot 0 \mu$ broad. The grain can be compared in shape, size and surface sculpture with the pollen grain of *Quercus robur* (Erdtman, 1843, p. 100, Fig. 192–93). Slide No. S.R. 8.

Tricolpites spm. 2.—Grain large, almost spherical, pale yellow. Pl. VI, Figs. 23 and 24; and Pl. VIII, Fig. 21 show the oblique polar view of the grain, which measures $53 \cdot 5 \mu \times 47 \cdot 6 \mu$. Only one flange is in focus (Pl. VI, Fig. 23) and measures $35 \cdot 7 \mu \times 37 \cdot 9 \mu$. Two longitudinal furrows, broad in the middle ($1 \cdot 2 \mu$) and narrow towards the end, run up to a little distance away from the pole. Pl. VI, Fig. 24 shows the same pollen grain, but with the other pole in focus. The two flanges and the third furrow are in focus. Pl. VIII, Fig. 21 shows all the three flanges but the nearest being in full focus. The two furrows (f) on either side of it are clearly seen and the third

furrow (*f*) is also seen hazily in the middle of the photograph. Exine 1·5 μ thick, smooth. Slide No. S.R. 11.

Tricolpites spm. 3.—Grain large, oblate, spheroidal, yellow. Oblique polar view (Pl. VI, Fig. 25; Pl. VIII, Fig. 22) measures 53·5 $\mu \times$ 47·6 μ. Furrows three, longitudinal, equatorial, stop a little distance from the poles. Flange 33·4 μ broad, outer surface convex. Exine thin about 1 μ; margin uneven. Surface sculpture positively reticulate, thickenings slightly sinuous, meshes broad towards the centre and smaller towards the periphery of the flange. Slide No. S.R. 11.

Tricolpites spm. 4.—Grain small, brown. Polar view (Pl. VI, Fig. 25; Pl. VIII, Fig. 23) subtriangular almost roundish, 35·7 μ diameter. Distal side of the flange slightly convex. Furrows longitudinal, equatorial, tapering to, but not reaching, the poles. Exine 3·5 μ thick, surface sculpture positively reticulate, meshes polygonal. It has not been possible to make out if the colpæ were also porate. If the grain turns out to be colporate, then a comparison can be made with *Pteropia* (Selling, 1947, p. 237, Figs. 470–76) and *Tetraplasandra* (Selling, 1947, p. 233, Figs. 441–44). But in the absence of the pores the grain can be compared in shape and surface sculpture with the comparatively smaller grains of *Hedera helix* (Erdtman, 1943, p. 68, Fig. 48) and *Olea europæa* (Wodehouse, 1935, p. 452, Pl. X, Fig. 3). Slide No. S.R. 2.

Trocolpites spm. 5.—Grain large, brown. Pl. VI, Figs. 27–28; Pl. VIII, Fig. 24 show the equatorial view (41·6 $\mu \times$ 48·8 μ), with the narrow longitudinal, furrow separating the two flanges seen at different foci. Exine 3·5 μ thick. Surface sculpture scrobiculate. The pits are very small. Pl. VI, Fig. 29; Pl. VIII, Fig. 25 show an oblique polar view of what appears to be the same type of grain, but slightly crushed out of shape. The thick exine, the convex outer surface of the flange (34·5 μ broad) and the surface scrobiculations are clearly seen. Slide Nos. S.R. 12 and S.R. 9.

Tetracolpites spm. 1.—Grain large, yellow. Polar view (Pl. VI, Fig. 30; Pl. VIII, Fig. 26) flattened round, 35·7 μ across. Colpæ four, deep, wedge shaped, equatorial, not reaching the poles. Flanges with slightly convex outer sides. Ektexine 1·0 μ thick, colourless, intexine 2·3 μ thick, darker in colour; surface sculpture finely scrobiculate. Equatorial view narrow and elongated and could not be sketched as the grain does not rest in that position. The grain can be compared with the slightly smaller pollen grain of *Fraxinus americana* (Erdtman, 1943, p. 114, Figs. 271–72). Slide No. S.R. 15.

Hexacolpites (Erdtman, 1947)

Hexacolpites spm. 1.—Grain large, light yellow. Equatorial view (Pl. VI, Figs. 31–32; Pl. VIII, Fig. 27) flat, probably due to pressure during fossilisation oblong. Polar diameter 53·5 μ, equatorial diameter 41·6 μ. Colpæ (*f*) six, seen at different foci in Pl. VI, Fig. 31–32. In Pl. VI, Fig. 31, three colpæ are clearly seen and the other three are clear in Plate VI, Fig. 32. Pl. VIII, Fig. 27 shows two colpæ clearly in the middle. Two others are partly seen one on each side. Colpæ equatorial, 26·1 μ long, very thin, slit-like and do not reach the poles. Ektexine less than 1 μ thin, intexine about 1·2 μ thick and dark in colour. Surface sculpture densely but minutely granular. Slide No. S.R. 8.

Hexacolpites spm. 2.—Grain small, brown, discoid, oblate. Polar view (Pl. VI, Fig. 33; Pl. VIII, Fig. 28) circular, 29·7 μ across. Furrows six, longitudinal, equatorial, not reaching the poles, wedge shaped, 7·4 μ deep, and 2·2 μ broad at the periphery of the grain. Exine 3·5 μ thick and smooth; surface sculpture slightly granular. Equatorial view narrow, elliptic, and could not be sketched as the grain does not rest in that position. The grain can be referred to *Nothofagus cliffortioides* (Erdtman, 1943, p. 100, Figs. 186–87) but with this difference, that in my grain the furrows are longer and not constricted at the equatorial part. Slide No. S.R. 4.

Hexacopites spm. 3.—Grain large, flat, yellow, discoid and peroblate. Polar view (Pl. VI, Fig. 34; Pl. VIII, Fig. 29) 35·7 μ across. Furrows six, equatorial, longitudinal 9·5 μ deep and 4·7 μ broad at the periphery of the grain. Exine thin, hyaline. Surface sculpture positively reticulate. This grain can be compared with the pollen of *Lycopus europæus* (Erdtman, 1943, p. 106, Fig. 233) as it agrees with it in size, shape and surface sculpture. Slide No. S.R. 10.

Hexacolpites spm. 4.—Grain large, discoid, brown, suboblate. Polar view (Pl. VI, Fig. 35; Pl. VIII, Fig. 30) round 38·1 μ across. Furrows six, 8·3 μ deep, narrow, longitudinal equatorial. Equatorial view narrow. Exine less than a micron thin. Surface sculpture densely granular giving a sort of negative reticulum-like appearance. Miss Cookson (1946) has described and figured some pollen *Nothofagus* from Australian Tertiary deposits. *Hexacolpites spm.* 4 comes well within the range of size of *Nothofagus* pollens described by Miss Cookson and also shows some resemblances in surface sculpture. Slide No. S.R. 12.

Septacolpites (Erdtman, 1947)

Septacolpites spm. 1.—Grain large, discoid, suboblate, yellow. Oblique polar view (Pl. VI, Fig. 36; Pl. VIII, Fig. 31) circular 35·7 μ across. Furrows

seven, equatorial, longitudinal 12·0 μ deep and 1·2 μ broad at the margin. Lobes 11·9 μ broad at the margin. Equatorial view narrow. Exine thin, smooth, uniform and hyaline; surface sculpture granular. The grain resembles closely in size, shape and surface sculpture the type referred above (*Hexacolpites spm.* 4), but differs from it in having seven colpæ. In view of this it has been kept separate. Slide No. S.R. 3.

Octacolpites (Erdtman, 1947)

Octacolpites spm. 1.—Grain large, brown, oblate. Polar view (Pl. VI, Fig. 37; Pl. VIII, Fig. 32) circular 47·6 μ across. Furrows 8·3 μ to 11·9 μ deep, longitudinal, equatorial and do not reach the poles. Equatorial view narrow. Exine thick; surface densely granular almost looking reticulate. Slide No. S.R. 3.

Triorites (Erdtman, 1947)

Triorites spm. 1.—(Pl. VI, Fig. 38; Pl. VIII, Fig. 33). A small, flat, yellow and badly preserved grain seen in polar view, 17·9 μ across, shows two pores (*p*) clearly at the angles. The third pore and the exine features are not very clear. But a comparison can be made with the grain of *Betula nana* (Erdtman, 1934, p. 74, Fig. 63). Slide No. S.R. 3.

Triorites spm. 2.—Grain small, flat, and yellow. Polar view (Pl. VI, Fig. 39; Pl. VIII, Fig. 34) triangular, sides straight 28·5 $\mu \times 27 \cdot 3 \mu$. Pores one at each angle, aspidate; subexineous thickening present 3·5 μ thick. Exine thin smooth; surface sculpture slightly granular. Equatorial view very elliptic. The grain can be compared with that of *Betula tortuosa* (Erdtman, 1948, Fig. 67, p. 74). A similar sized grain but with a very slight difference in appearance has been described from Mohgaon Kalan cherts by Mrs. Chitaley (1950, p. 379, Fig. 22, Text-Fig. 23) under the name *Triorites* (*Betulacidites* spm. Slide No. S.R. 3.

Triorites spm. 3.—Pl. VI, Figs. 40–42 show a type of pollen which is yellow and of variable size. The best preserved of these is described. Polar view (Pl. VI, Fig. 40; Pl. VIII, Fig. 35) flat, circular, 40·4 $\mu \times 42 \cdot 8 \mu$ across. Equatorial view could not be made out. Pores three, aspidate, slightly elongated, bounded by subexineous thickening. Exine 2·4 μ thick; surface yellow, granular. This grain resembles the pollen of *Carpinus betulus* (Edtman, 1942, p. 76, Figs. 76–78) which has also been described from the Palana lignites, Type No. 17 (Rao and Vimal, 1951). Pl. VI, Figs. 41 and 42 show the polar and oblique polar views respectively of two different grains of the same species but slightly bigger. Slide No. S.R. 3.

DISCUSSION AND CONCLUSION

Tertiary coals of India have not received much attention, although some work has been published on them by Gosh, A.K. (1941) from Assam coal fields, Sen, J. (1948) also from Assam, Rao and Vimal (1950) from Palana, Jacob and Jacob (1950) from Cuddalore, S. Arcot, Madras. Mrs. S. D. Chitaley (1950–51) has described some microfossils from the Eocene Inter-Trappean Cherts of Madhya Pradesh. The present paper deals with the description of some of fossil pollen and spores from the Tertiary coal of Dandot, Salt Range, Pakistan.

A few of the pollen types described in this paper resemble those found in other Eocene floras like the Deccan traps of India, the Palana lignites, Green River oil shales of Colorado and Utah, etc.

Although the coal is of the same age as that of Palana, it differs from it in microfossil contents. Pteridophitic spores which have not so far been observed in Palana coal, are very abundant here. Gymnospermic pollen grains have not been observed either in the Palana coal or in the Dandot coal. Some of the angiospermic pollen grains are identical with the grains found in Palana coal, *e.g.*, *Nothofagus*, *Potamogeton*, *Corylus americana*, *Betula tortusa*, *Carpinus betulus*; some of these types are common to both the Palana lignites and the Dandot coal. But a striking difference between these two lignites is the preponderance of pollen in Palana lignites, almost to the exclusion of pteridophitic spores—atleast as far as observed; while the Dandot coal shows a mixed flora—of Pteridophytes and Angiosperms. Wodehouse (1935, p. 357) has pointed out that pollination by wind tends to bring about a reduction of the exine with an attendant loss or reduction of its structure such as furrows, pores and sculpturing. Fægri and Iverson (1951) also think that anemophilous pollen are smooth while zoogamous pollen have a sculptured surface. Judging by this criterion it may be pointed out that most of the pollen and spores described from Dandot in this paper appear to be sculptured and so possibly zoogamous.

The pollen grains described in this paper and compared as far as possible, include pollen of trees (*Betula*, *Carpinus*, *Corylus*, *Nothofagus*) and aquatic herbs (*Potamogeton*). But the majority of pollen and spores could not be satisfactorily compared. It is therefore inadvisable to make any definite inferences about the flora in general.

SUMMARY

The paper deals with fossil spores and pollen recovered from lignites (Eocene) of the Dandot coalfields, West Punjab (Pakistan). Ten different kinds of spores referred to the form genus *Trilites* and twenty types of pollen

grains, classed under *Nonaperturites*, *Monocolpites*, *Dicolpites*, *Tricolpites*, *Tetracolpites*, *Hexacolpites*, *Septacolpites*, *Octacolpites* and *Triorites*, have been described here. Some of the spores resemble those of *Ophioglossum falcatum*, *O. vulgatum*, *Lycopodium alpinum*, *Schizostege Lydgatei*, *Doryopteris decora*, *Selaginella deflexa*; some of the pollen on the other hand compare with those of *Potamogeton natans*, *Quercus robur*, *Hedera helix*, *Olea europæa*, *Pteropia*, *Tetraplasandra*, *Fraxinus americana*, *Nothofagus cliffortioidis*, *Lycopus europæus*, *Betula nana*, *B. tortuosa* and *Carpinus betulus*. Most of these pollen so far as compared, appear to belong to trees and have a sculptured outer coat which according to some is a sign of zoogamous pollen.

A few of the pollen grains described here, have also been reported from other, probably Eocene strata, like Palana lignites, Eoecne beds of Green river (Colorado, Utah, U.S.A.), Tertiary deposits of Australia and Intertrappean cherts of Mohgaon Kalan, Madhya Pradesh, India.

ACKNOWLEDGEMENTS

I wish to express my grateful thanks to Dr. A. R. Rao, Reader in the Botany Department, under whose valuable guidance this work has been carried on. My thanks are also due to Dr. R. C. Misra for the loan of material and to Dr. K. M. Gupta for the loan of some literature. I am greatly indebted to the authorities of the Birbal Sahni Institute of Palæobotany, whose library facilities I have made full use of. This investigation has been carried out with the help of a Government of India Scholarship and I am thankful for this financial help. Lastly, my thanks are also due to Prof. S. R. N. Rao, Head of the Geology Department, for his kind encouragement during the course of this work.

REFERENCES

1. Chitaley, Mrs. S. D. .. "Microflora of the Deccan Intertrappean Cherts," *Palæobotany in India*, No. 7, *Journ. Ind. Bot. Soc.*, 1950, 29–30.
2. ——— .. "Fossil Microflora from Mohgaon Kalan Beds of Madhya Pradesh, India," *Proc. Nat. Inst. of Sciences of India*, 1951, **17**, No. 5.
3. Cookson, I. C. .. "Pollen of Nothofagus Blume from Tertiary Deposits in Australia," *Proc. of the Linnean Soc. of New South Wales*, 1946, **71**, Parts 1–2.
4. Erdtman, G. .. *An Introduction to Pollen Analysis*, Waltham, Mass, U.S.A., 1943.
5. ——— .. "Suggestions for the classification of fossil and recent pollen grains and spores," *Svensk Botanisk. Tadskrift.*, 1947, **Bd. 41, H. 1.**
6. Fageri, K. and Iverson, J. *Text book of Modern Pollen Analysis*, Ejnar Munksgaard, Copenhagen, 1950.

7. Fisher, H. .. "Beitrage zur vergleichen den Morphologie der Pollen-Korner," *Dissertation Breslau*, 1890, **69**, p.pl. **1.**

8. Ghosh, A. K. .. "Fossil Pollen in the Tertiary Rocks of Assam," *Science and Culture*, 1941, **6.**

9. Hofmann, E. .. "Neus von der Pollenanalyse," *Schriffen des Vereines zur Verbreitung naturwissenschafflicher Kenntnisse i. Wien.*, 1950.

10. Ibrahim, A. C. .. "Sporenformen des Aegirhorizonts des Ruhr-Reviers," *Diss. K. Triltsch. Wurzburg*, 1933.

11. Jacob, K. and Mrs. Chinna Jacob "On Spores and Pollen Grains from the Tertiary Lignite of Cuddalore, S. Arcot, India," *7th International Botanical Congress, Stockholm*, 1950.

12. Kirchheimer, F. Halle .. *Ein Beitrag zur Kenntnis Von Pollenformen der Eozanbraun-kohle des Geiseltales*, 1931.

13. ———— .. "Uber pollen aus der jungtertiaren Braunkohle von Salzhausen (oberhessen)," *Neu. Jahr. Mineralogie*, 1932, **67**, 304–12.

14. Knox, E. M. .. "Spore Morphology in British Ferns," *Trans. and Proc. of the Bot. Soc. of Edinburgh*, 1951, **35**, Part **4.**

15. La Touch, T. D. .. "Report on the occurrence of coal at Palana village in Bikaner State," *Rec. G.S.I.*, 1897, **30**, Part 3.

16. Naumova .. "The Spores and Pollen of the Coals of U.S.S. R.," *Abstract XVII International Geol. Cong., U.S.S.R.*, 1937, 60–62.

17. Potonie, H. .. *Die Flora des Rothliegenden Von Thuringen*, Berlin, 1893.

18. Potonie, R. .. "Pollenformen der miocenen Braunkohle," *Sitzungsber Ges. Nat. Freunde*, Feb. 17, 1931.

19. ———— .. "Zur Mikroskopie der Braunkohle, Tertiare, Sporen und Blutenstaub-formen," *Braunkohle*, 1931, **30**, 554–56, f. 1–15.

20. Raistric and Simpson .. "The microspores of some Northumberland coals and their use in the correlation of coal seams," *Inst. Min. Eng. Trans.*, 1933, **85**, 225–35.

21. R. A. Couper and others *Terminology and Nomenclature for Fossil Pollen and Spores.*

22. Rao, A. R. and Vimal, K. P. "Plant Microfossils from Palana Lignites (? Eocene), Bikaner," *Curr. Sci.*, March 1950, 80–84.

23. ———— .. "Tertiary Pollen from Lignites from Palana," *Proc. Ind. Sci. Congr.*, Bangalore, 1950.

24. Schose Bodmer, H. .. "The Influence of Nutrition upon Pollen Grains in *Lytheum solicria*," *Journ. Genetics*, 1940, **40**, 393.

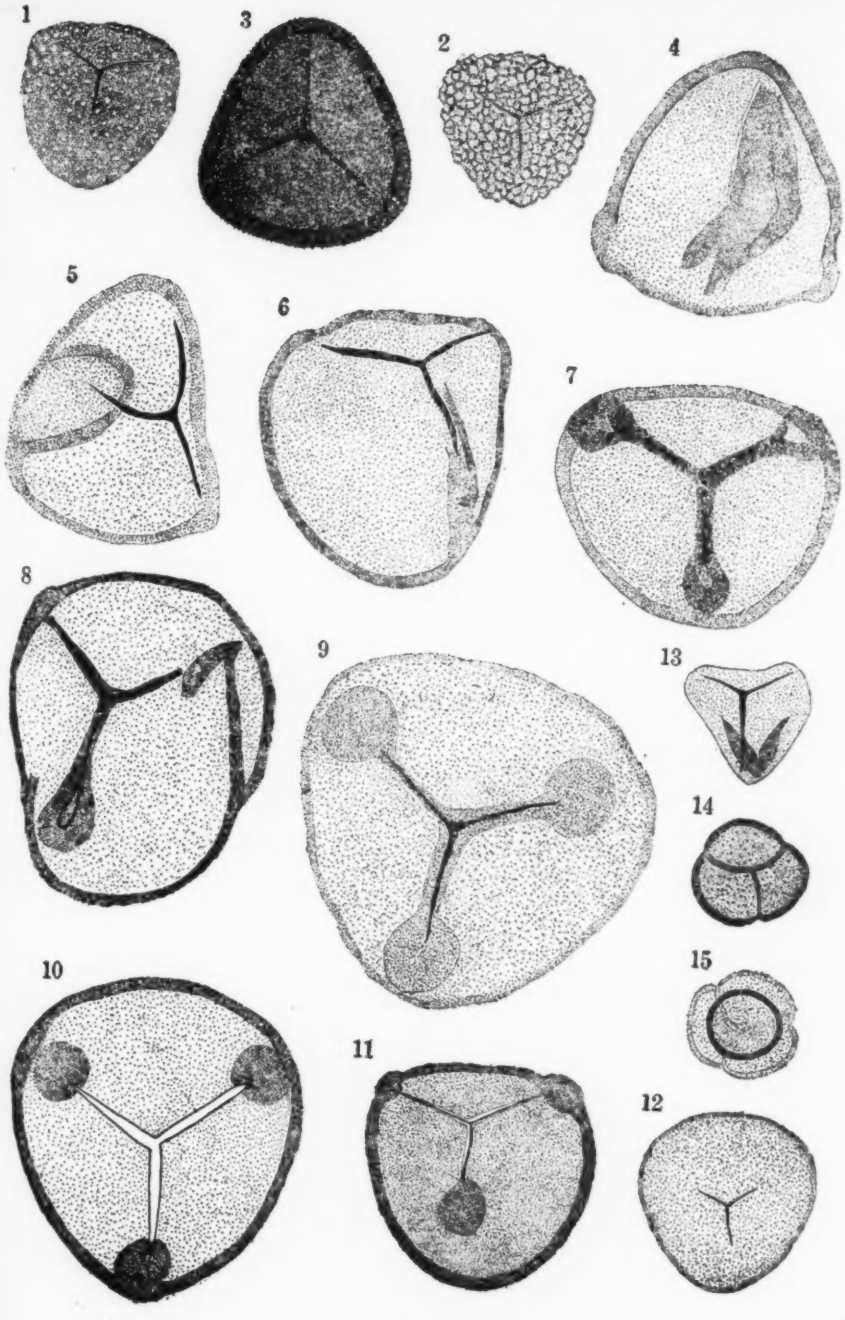
25. Selling, O. H. .. *Studies in Hawaiian Pollen*, Part I, 1946.

26. ———— .. *Studies in Hawaiian Pollen Statistics*, Part II, published by the Bishop Museum, Honolulu, Hawaii, 1947.

27. Sen, J. .. "Microfossils of Assam Coalfields—I. Coal seam at Latryngen and the Age of the Cherra Sandstone,"*Bull. Bot.Soc.*, Bengal, 1948, **2**, 94–101.

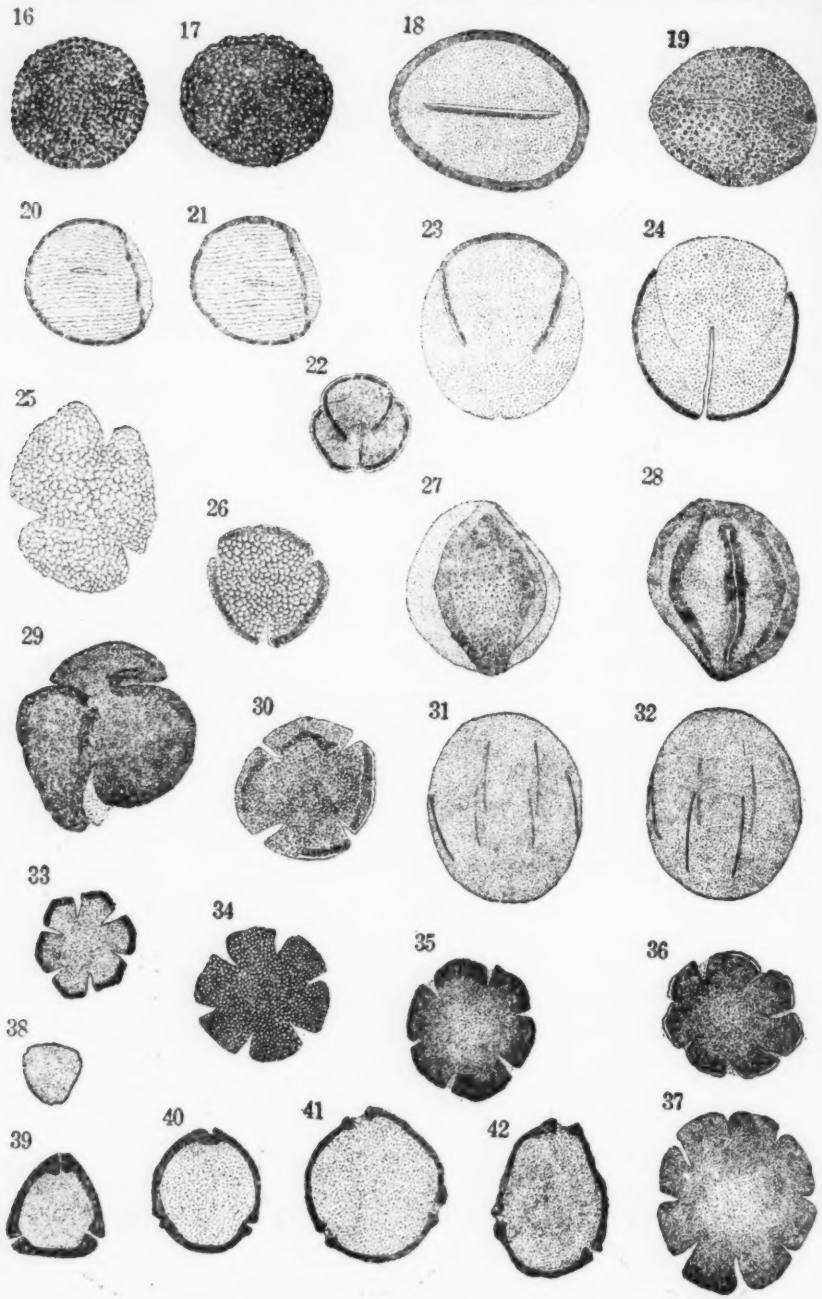
28. Wodehouse, R. P. .. "Tertiary Pollen," *Bullettin of the Torrey Club*, 1933, **60.**

29. ———— .. *Pollen Grains*, McGraw Hill Book Co. Inc., New York 1935,

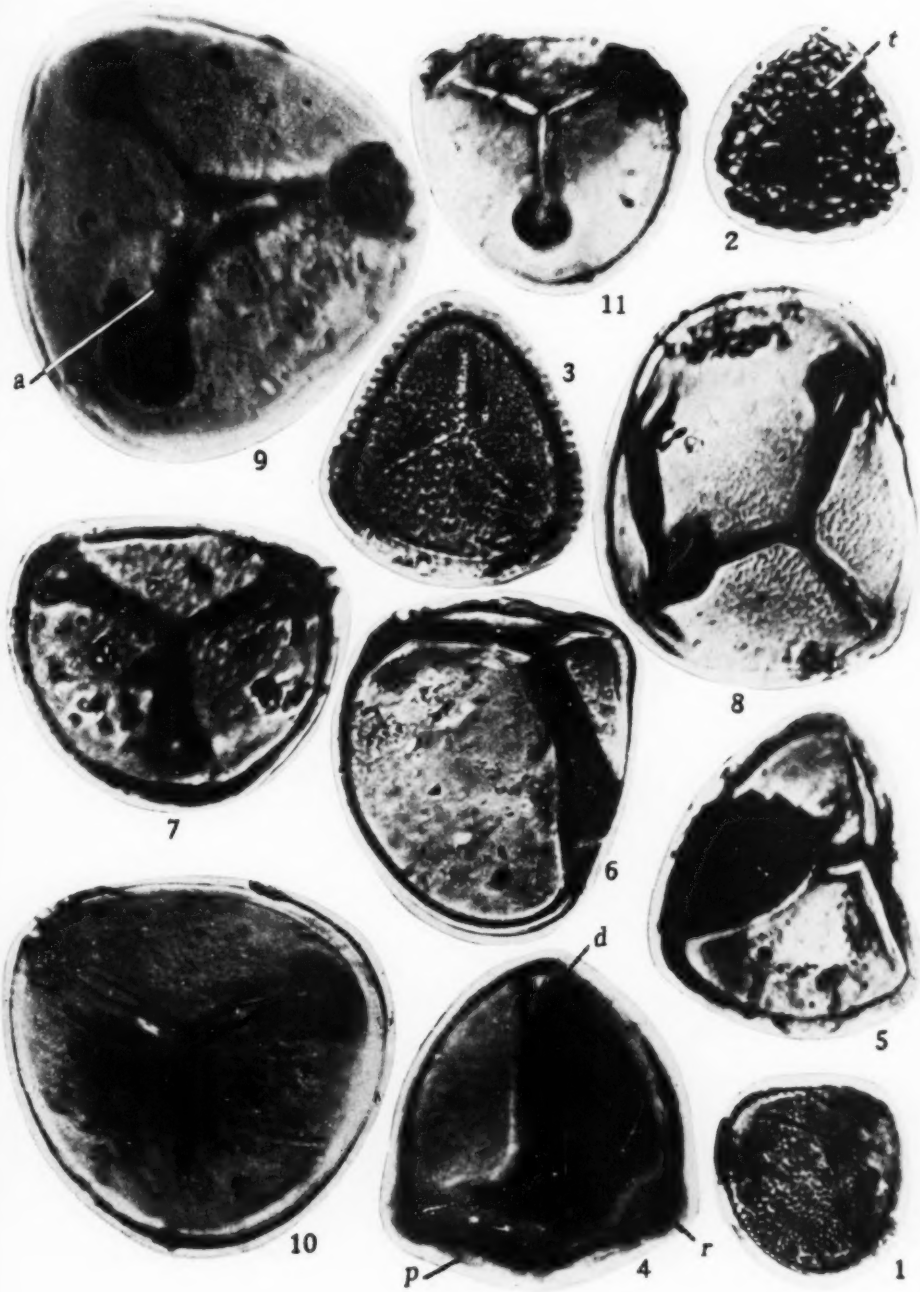


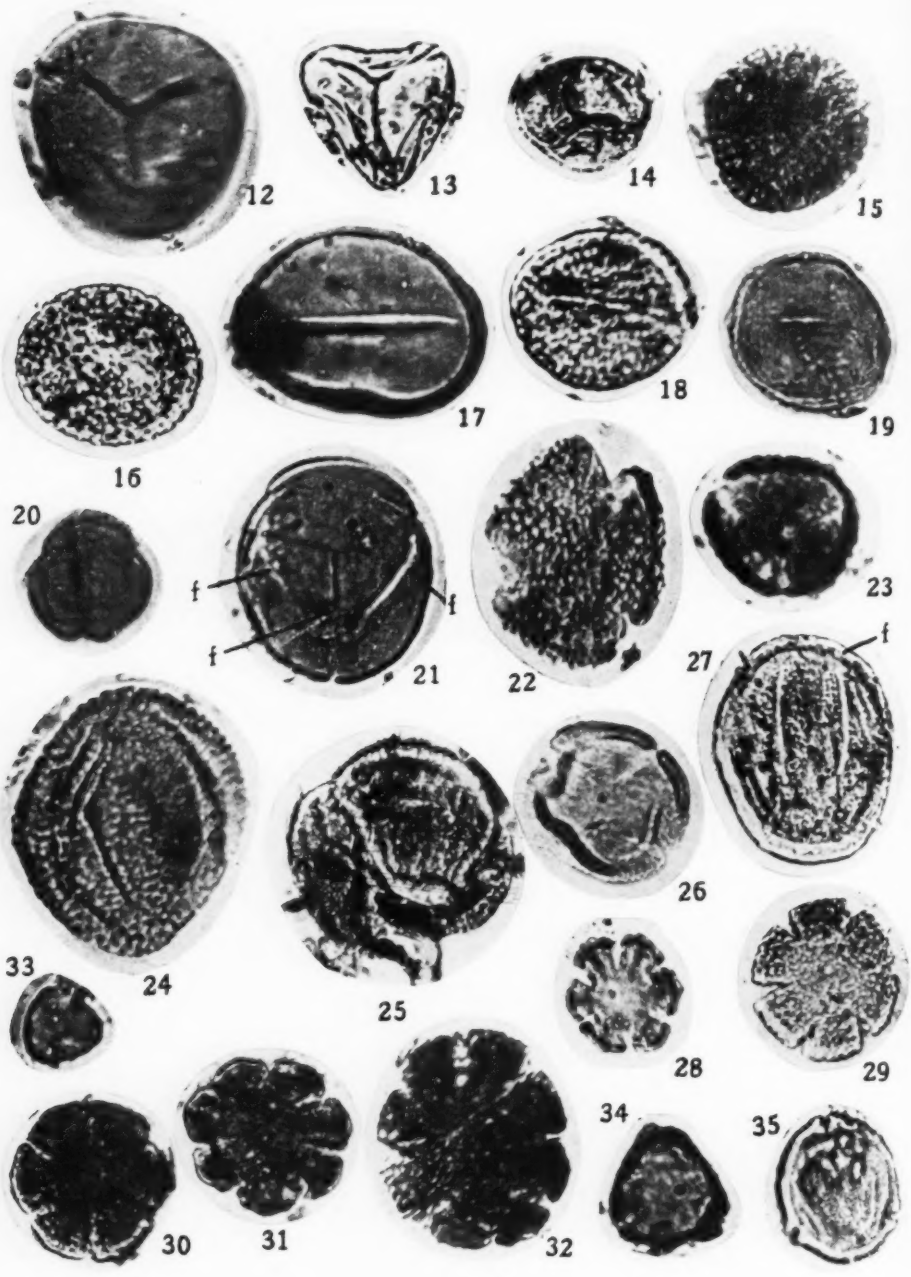
Trilites. 1–15.

Explanation in Text. All figures magnified 650 times.



Nonaperturites, 16–17 ; *Monocolpites*, 18–19 ; *Dicolpites*, 20–21 ; *Tricolpites*, 22–29 ; *Tetracolpites*, 30 ; *Hexacolpites*, 31–35 ; *Septacolpites*. 36 ; *Octacolpites*, 37 ; *Triorites*, 38–42.
Explanation in Text. All figures magnified 650 times.


a
9
11
t
2
3
8
7
6
5
d
10
p
4
r
1


12
13
14
15
16
17
18
19
20
f
f
f
21
22
23
27
f
24
25
26
33
28
29
30
31
32
34
35

Explanation of Photographs

All photographs are from untouched negatives and unless otherwise stated are magnified 587·2 times.

1. ***Trilites*** spm. 1, Oblique polar view. 2. ***Trilites*** spm. 2, Proximal view, *t*, trilete mark. 3. ***Trilites*** spm. 3, Proximal view. 4. ***Trilites*** spm. 4, Equatorial view, *r*, rim ; *d*, distal side ; *p*, proximal side. 5. ***Trilites*** spm. 4, Proximal view. 6. ***Trilites*** spm. 4, Oblique view. 7. ***Trilites*** spm. 5, Proximal view, *t*, triradiate mark ; *s*, slit. 8. ***Trilites*** spm. 5, Proximal view. 9. ***Trilites*** spm. 6, Proximal view. 10. ***Trilites*** spm. 6, Proximal view. 11. ***Trilites*** spm. 7, Proximal view. 12. ***Trilites*** spm. 8, Proximal view. 13. ***Trilites*** spm. 9, Proximal view. 14. ***Trilites*** spm. 10, Tetrad. 15. ***Subpilonapites*** spm. 1, Probably polar view. 16. ***Reticulonapites*** spm. 1, Probably equatorial view. 17. ***Monocolpites*** spm. 1, Equatorial view. 18. ***Monocolpites*** spm. 2, Probably equatorial view. 19. ***Dicolpites*** spm. 1, Perhaps equatorial view. 20. ***Tricolpites*** spm. 1, Oblique polar view. 21. ***Tricolpites*** spm. 2, Oblique polar view, *f*, furrows. 22. ***Tricolpites***, spm. 3, Oblique polar view. 23. ***Tricolpites*** spm. 4, Polar view. 24. ***Tricolpites*** spm. 5, Equatorial view. 25. ***Tricolpites*** spm. 5, Oblique polar view. 26. ***Tetracolpites*** spm. 1, Polar view. 27. ***Hexacolpites*** spm. 1, Equatorial view. *f*. furrows. 28. ***Hexacolpites*** spm. 2, Polar view. 29. ***Hexacolpites*** spm. 3, Polar view. 30. ***Hexacolpites*** spm. 4, Polar view. 31. ***Septacolpites*** spm. 1, Oblique polar view. 32. ***Octacolpites*** spm. 1, Polar view. 33. ***Triorites*** spm. 1, Polar view. 34. ***Triorites*** spm. 2, Polar view. 35. ***Triorites*** spm 3, Polar view.

OXYGEN CONSUMPTION AND VIABILITY OF *CHANOS CHANOS* (FORSKÅL) IN RELATION TO SIZE*

By R. Viswanathan and P. R. S. Tampi
(*Central Marine Fisheries Research Station, Mandapam*)

Received July 29, 1952
(Communicated by Dr. N. K. Panikkar, F.A.SC.)

Introduction

The importance of the size factor in metabolic studies on fish has been emphasised by Rubner (1924) who found that the oxygen consumption in the pike and in the tench was correlated exponentially with the body weight of the fish. More recently, Busnel, Drilhon and Raffy (1946) have investigated the oxygen consumption of the fry and alevins of the rainbow trout in waters of varying salinity; their results indicate that the salinity of the external medium is a second decisive factor influencing respiration.

It is obvious that the relationship between size and metabolic activity is highly significant in the transport of live fish. Estimates of the safe limits of numbers of fish that can be transported in limited volumes of water can be based on trial and error methods as has been done in *Chanos chanos* (Ganapathi, *et al.*, 1950). Alternatively, charts can be prepared, based on standard experimental data, from which it should be possible to read off information on the viability of the fish under different environmental conditions. The preparation of such charts would involve, among other things, a knowledge of the rate of variation of oxygen consumption with the size of the fish and a method for properly differentiating size effects from salinity effects. The present account describes an attempt along these lines to analyse the problems of transport of live fry and fingerlings of *Chanos*.

Material, Methods and Results

I. Length, Weight and Oxygen Consumption in Chanos

(*a*) The gear employed in obtaining the different sizes of fish are known to influence the length-weight equation (*e.g.*, Le Cren, 1951). They will therefore be first described: (i) A mosquito-net cloth was used for collecting fry of length 16–40 mm. from the tidal creeks at Chinnapalam (Gulf of

* Published with the permission of the Chief Research Officer, Central Marine Fisheries Station, Mandapam.

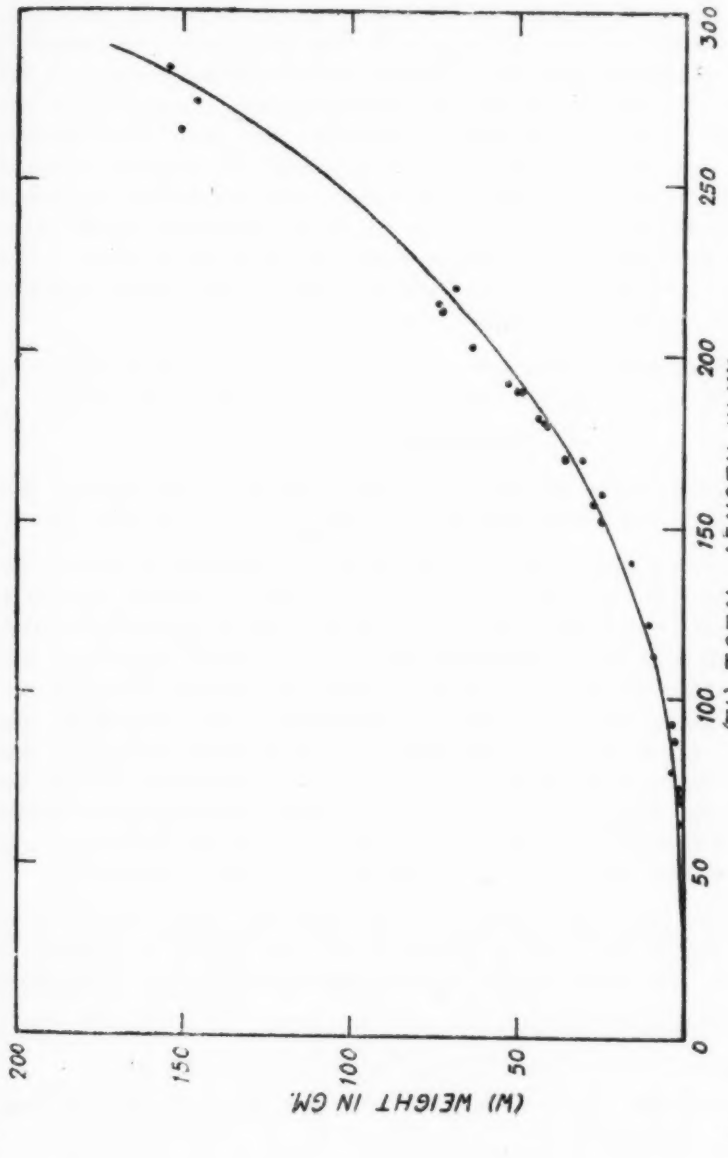


FIG. 1. Total length-weight relationship of *Chanos chanos*. The curve is the graph of the equation $W=0{\cdot}000034626\ (T.L.)^{2{\cdot}673}$. The dots represent the observational data.

Mannar) where they swarm in large numbers; (ii) Fingerlings of about 100 mm. length were caught by dragging a scareline along the creeks and allowing the fish to jump into a piece of cloth held close to the scareline; (iii) Fish of larger size but below 200 mm. in length were obtained at Horse-shoe Bay, Pamban, by the use of "Kalamkattivalai", a circular gill net, $\frac{1}{2}$" sq. mesh, supported on stakes at high tide; and (iv) Fish longer than 200 mm. were caught in drift nets operated within the chain of islands in the Gulf of Mannar. The total length of the fish was measured (to the nearest mm.) from the tip of the snout to the end of the dorsal lobe of the caudal fin. The fish were weighed after removing the water on the body surface by the use of filter-paper. The fry were weighed to the nearest milligram and the larger fish to the nearest decigram.

Fig. 1 gives the total length-weight curve of *Chanos* up to a total length of 300 mm. of the fish. The curve (which is the graph of the equation

$$W = 0{\cdot}000004626\ L^{3{\cdot}07\pm24}, \qquad (1)$$

where W = weight of the fish in gm. and L the total length in mm.) was fitted to the observational data by the regression method of least squares.

(*b*) For the determination of the oxygen consumption of *Chanos*, the fry or fingerling was starved overnight in the selected medium after which it was placed for 1–2 hours in a definite volume (290 ml. per fry or 1900 ml. per fingerling) of water contained in an Erlenmeyer flask. A seal of liquid paraffin above the surface of water prevented the diffusion of oxygen and carbon dioxide from and into the atmosphere. The temperature and dissolved oxygen content of the water were determined both before and after the introduction of the fish into the water. Winkler's method was used for the determination of dissolved oxygen. The weights of the fry were determined in a chemical balance and those of the fingerlings calculated from their lengths by the use of the length-weight equation (1).

Data on the oxygen consumption of *Chanos* are summarised in Fig. 2. For the fish in tap water (of salinity 1 part per 1,000 and temperature 28–31° C.), the weight-oxygen consumption relationship is satisfactorily described by the equation

$$Q = 1{\cdot}829\ w^{0{\cdot}8342}, \qquad (2)$$

where Q is c.mm. of oxygen consumed per hour by fish weighing w mgm.

From equations (1) and (2), we get

$$Q = 0{\cdot}02063\ L^{2{\cdot}563} \qquad (3)$$

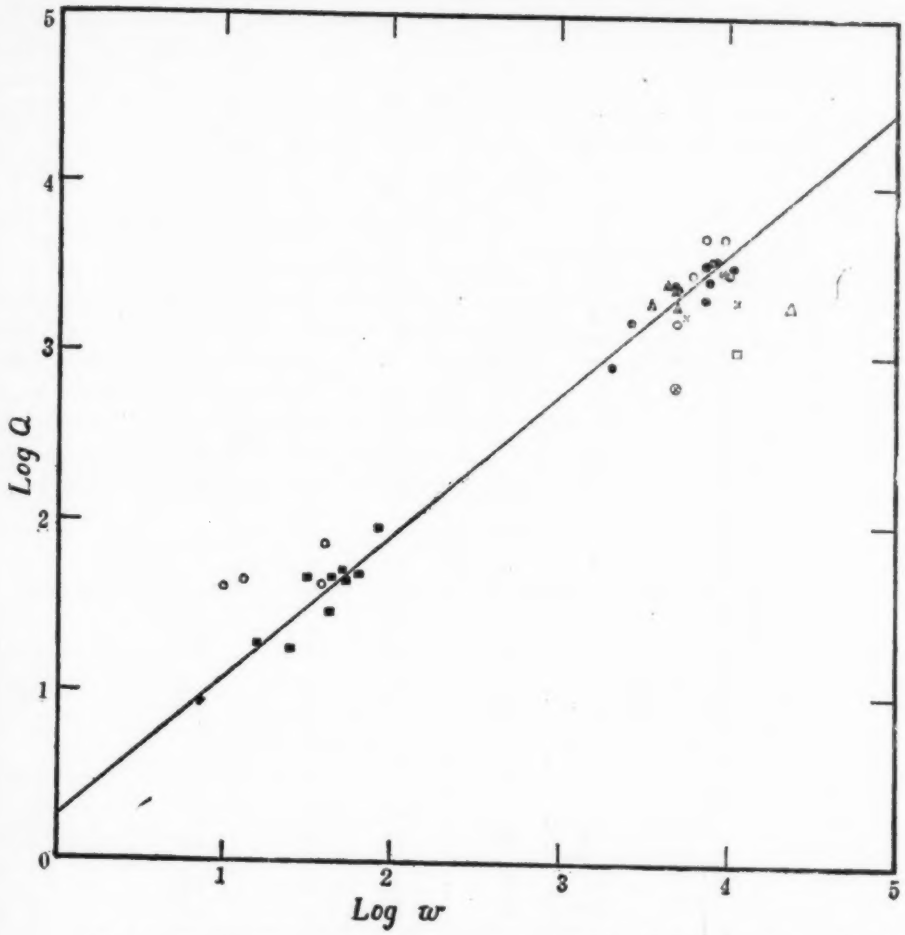


FIG. 2. Oxygen Consumption of *Chanos*. w = Weight of fish in mgm., Q = µl. oxygen consumed per hour. The straight line is the graph of the equation Log Q = 0·2622 + 0·8342 Log w. (1) Tap water, 28° – 31° C ●; 30° C ▲; 25° C ■; 25° C ×; (2) 25% sea water at 25° C ×; and (3) sea water, 30° C O; 27° C △; 25° C □.

II. Respiration of Chanos *Fry in a Noncirculating Volume of Water*

Chanos fry were preacclimatised to the experimental media for a day. 200 ml. of the test media (in thermal equilibrium with the atmosphere, temperature 30–31° C.) were taken in 500 ml. bottles and the fry were transferred to the bottles as quickly as possible by the use of a hand net. The bottles were then loosely stoppered, a little space being left for the entry

TABLE I

Survival of Chanos *fry in noncirculating volume of water in open containers*

Medium	Total number of fry	Length of fry				Weight of fry g.	Ml. (initial) oxygen per g. fish (= X)	Time in hrs.										Per cent. survival of fry
		No. of fry in sample	Range mm.	Mean mm.	S.D.			1·0	1·5	2·0	2·5	3·0	3·5	4·0	4·5	5·0	5·5	6·0	24	48
Sea water ($Cl‰$ = 17·9. Dissolved Oxygen = 3·37 ml./l.)	5	15	23–28	24·8	1·2	0·446	1·511	80	80	80	80	80	80	80	80	80	80	80	80	..
	20					1·795	0·375	100	85	85	85	85	85	85	85	85	85	83	20	..
	5	..	..	..	..	..	..	100	100	100	100	100	100	100	100	100	100	100	100	100
	20	..	..	..	..	..	..	40	35	30	..	..	15	15	10	..	5	0	0	
	5	8	22–41	29·8	7·6	0·780	0·864	100	100	100	100	100	100	100	100	100	100	100	100	100
	20					3·347	0·201	75	70	..	..	60	55	45	..	..	40	0	0	
Tap water ($Cl‰$ = 1·07, Dissolved Oxygen = 3·37 ml./l.)	5	21	15–32	23·1	3·5	0·358	1·883	100	100	100	100	100	100	100	100	100	100	100	100	100
	21					1·614	0·418	100	100	95	95	95	95	95	95	95	95	95	95	..
	5	..	..	..	..	..	..	100	100	100	100	100	100	100	100	100	100	100	100	100
	20					..	..	100	100	100	100	95	95	95	95	95	95	95	95	80
Tap water	10	10	31–50	32·8	5·8	2·314	0·1845	..	..	90	..	..	..	..	..	..	..	..	..	..
	20	20	22–50	36·8	9·2	7·007	0·0598	..	..	15	..	..	..	..	..	..	..	..	..	..

of air into the bottle. The lengths of the fry were measured at the conclusion of the experiments. Where the lengths of all the fry couldn't be measured, the mean length of a suitable sample was taken to represent that of the population experimented upon. For the calculation of the weight of the fry, the length-weight equation (1) was used. The results are given in Table I.

III. Asphyxiation Experiments on Chanos *Fry*

The methods were similar to those used in the experiments described in Section II excepting that the bottles were completely filled with the medium and fully stoppered. Observations of mortality were made at intervals of 30 minutes. The per cent. survival of the fry was plotted against the time of experiment and the median survival time, *i.e.*, the time in which 50% of the fry die, was read off from the graphs. In Tables II and III are given the correlation coefficients and regression equations relating to the median survival time and the ml. (initial) oxygen per gm. fish.

TABLE II

Mortality of Chanos *fry under conditions of asphyxiation in sea water*

($Cl‰$ = 18·46, D.O. = 2·93 ml./l.)

Volume of water ml.	Total no. of fry	Length of fry				Weight of fry g.	Ml. (initial) oxygen per g. fish (= X)	Median survival time (mts.) = Y
		No. of fry in sample	Range mm.	Mean mm.	S.D. mm.			
586	6	6	14–25	17·2	4·1	0·210	8·177	..
534	14	9	14–27	19·2	4·5	0·627	2·496	30
570	17	17	14–25	17·7	3·2	0·594	2·812	27
586	26	26	14–25	18·0	3·9	1·004	1·710	105
566	8	8	13–22	15·5	2·8	0·186	8·917	..
586	12	12	14–25	17·1	3·8	0·393	4·369	117
548	18	18	14–25	19·4	3·3	0·921	1·956	135
572	35	35	11–24	18·0	3·4	1·281	1·308	102
560	6	6	13–20	17·3	4·4	0·210	7·814	384
584	12	11	13–23	18·1	3·6	0·447	3·828	240
560	23	22	13–27	17·1	3·2	0·736	2·229	108
562	22	22	14–25	19·1	3·7	0·957	1·703	153

Correlation coefficient $r_{xy} = +0·7778$

Regression equations:

$X = 3·023 + 0·01155\,(Y - 140·1)$

$d_x = 0·01155\,d_y$

$Y = 140·1 + 42·02\,(X - 3·023)$

$d_y = 42·02\,d_x$

TABLE III

Mortality of Chanos *fry under conditions of asphyxiation in hard tap water*

(Cl‰ = 0·98, D.O. = 3·37 ml./l., Ca = 5·6 mE./l.)

Volume of water ml.	Total no. of fry	Length of fry				Weight of fry g.	Ml. (initial) oxygen per g. fish (=X)	Median survival time (mts.) = Y
		No. of fry in sample	Range mm.	Mean mm.	S. D. mm.			
586	7	6	16–27	23·2	4·0	0·536	4·056	399
534	13	13	16–26	21·9	3·7	0·852	2·326	222
570	19	19	15–25	18·4	3·6	0·757	2·794	249
586	23	23	17–28	20·8	3·4	1·293	1·682	165
566	5	5	15–25	21·0	4·1	0·291	7·216	.
586	13	13	15–25	17·8	3·8	0·477	4·588	24
548	15	15	15–26	20·9	4·4	0·893	2·277	171
572	25	25	14–27	18·7	4·2	1·055	2·011	171
560	5	5	15–19	16·0	1·7	0·119	17·460	..
584	14	14	15–27	17·6	3·3	0·486	4·439	467
560	17	17	13–20	16·9	1·5	0·482	4·311	387
562	22	22	14–27	18·8	3·0	0·947	2·202	201

Correlation coefficient $r_{xy} = + 0·3636$

Regression equations:

$X = 3·071 + 0·0031\ (Y - 245·6)$

$d_x = 0·0031\ d_y$

$Y = 245·6 + 42·56\ (X - 3·071)$

$d_y = 42·56\ d_x$

DISCUSSION

It may be seen from Figs. 1 and 2 that there is a close fit between the observed and calculated values over the entire range of total length studied (0–300 mm.). This indicates the absence in this range of sharp changes in the length-weight relationship and in the physiological behaviour such as would be caused, for instance, by the onset of maturity. The immaturity in 300 mm. long fish, suggested by this good fit, is in accord with independent observations on the gonadic condition of fish 500 mm. long which revealed that fish of even this size were immature. When applied to the only specimen of adult *Chanos* we could get, equation (1) showed a discrepancy of 2,420 gm. between the observed and calculated weights [total length of fish with spent ovaries, 1,240 mm.; standard length, 1,000 mm.; observed weight, 12,250 gm.; weight calculated from equation (1), 14,670 gm.].

In regard to the oxygen consumption of *Chanos*, it is seen from Fig. 2 that for the same salinity of the water, the oxygen consumption in the case of fish kept at 25–27° C. (during the colder months) is less than that of fish (at 28–31° C.) experimented on during the summer months. The differ-

ences between the means of oxygen consumption values in tap water and in sea water are, however, not highly significant [t (for fingerlings at 30° C.): calcd., 0·12; from Tables, 2·365 for P = 0·05; t (for fry at 28° (tap water) and at 30° C. (sea water): calcd., 2·34; from Tables, 2·18 for P = 0·05 and 2·68 for P = 0·02]. A similar negligible influence of salinity has also been observed in the case of another metabolic characteristic, namely, the heat resistance of *Chanos* fry (Panikkar, Tampi and Viswanathan, unpublished).

The usefulness of the equations (1–3) in the study of the transport of live *Chanos* remains to be considered. Equations (4–7), derived from equations (1–3), deal with the relationship between the time of survival of the fish, the number and length of the fish and the volume of the external medium. Two simplifying assumptions have been made: (i) All the "first deaths" (Keys, 1931) are simultaneous at the critical oxygen tension. The classification of deaths of fish under conditions of asphyxiation into "first deaths" and "second deaths" was suggested by Keys. Since the mortality of fish during transport in a noncirculating volume of water is primarily due to lack of adequate oxygen and consequent asphyxiation, the use of Keys' concept of "first deaths" in the present case appears to be relevant. (ii) The net rate at which oxygen enters the water is negligible in comparison with the rate at which oxygen is consumed by the critical numerical limits of fish under transport.

Let the initial oxygen content of V ml. of the medium be X_1; let n_1 be the number of fish in V ml. of the medium; l_1, the average length in this batch of fish; r_1, the amount of oxygen consumed per hour per fish; and t_1, the time of survival, *i.e.*, the time in which there is a chosen level of mortality among the fish. If n_2, l_2, r_2 and t_2 are the corresponding characteristics of a second batch of fish and k the exponent in the length-weight equation, we have at the critical oxygen tension,

$$X_1 - n_1 t_1 l_1^k = X_1 - n_2 t_2 l_2^k \qquad (4\text{ i})$$

$$X_1 - n_1 t_1 r_1 = X_1 - n_2 t_2 r_2 \qquad (5\text{ i})$$

$$t_2 = t_1 (n_1/n_2) (l_1/l_2)^k \qquad (4)$$

$$\text{and } t_2 = t_1 (r_1/r_2) (n_1/n_2) \qquad (5)$$

Where the second batch of fish is kept in mV ml. of water, m being a dilution factor, we have, at the critical oxygen tension,

$$X_1 - n_1 t_1 l_1^k = (mX_1 - n_2 t_2 l_2^k)/m \qquad (6\text{ i})$$

$$X_1 - n_1 t_1 r_1 = (mX_1 - n_2 t_2 r_2)/m \qquad (7\text{ i})$$

$$t_2 = mt_1 (n_1/n_2) (l_1/l_2)^k \qquad (6)$$

$$\text{and } t_2 = mt_1 (n_1/n_2) (r_1/r_2) \qquad (7)$$

Equations (4–7) could be used for the compilation of charts (see Appendix I) to indicate the number of fish which could be transported in known volumes of water. Adjustments of the volume of water and the frequency of change of water could be referred to experimental observations on the survival of fish combined with routine determinations on the lengths of the fish in random samples from the various batches of fish. It is proposed to test the utility of equations (4–7) as and when fish fry and fingerlings become available.

The data contained in Tables I–III clearly indicate the need for taking into consideration the weight of the respiring fish in its relation to the initial oxygen content of the medium. In Table I, the median survival time values are respectively 3·5 hours and 6–24 hours for values of X corresponding to 0·20 and 0·38 in sea-water. For higher values of X in sea-water (1·51 and 0·86), the survival in 24 hours is proportionately higher (80 and 100% respectively). Thus it is seen that a comparison of the viability of the fish under different environmental conditions is facilitated by the use of the factor x; while a mere grouping of the fish in terms of their length ranges or number in a given volume of water is not directly helpful in interpreting the experimental results. The advantages of using the factor X are, however, only to be expected in view of the very fundamental relationship between weight and oxygen consumption.

The difference between the correlation coefficients in Tables II and III is not significant at the probability level of 0·05 (Paterson, 1939), indicating thereby that salinity has no influence on the viability of the fry under conditions of asphyxiation. In a non-circulating volume of water in open containers, on the other hand, the mortality of the fry is significantly less in hard tap water than in sea water, for the same value of X (= 0·4) in both the salinities.

SUMMARY

Equations have been derived interrelating length, weight and oxygen consumption in *Chanos chanos*. The uses which these equations can be put to in the study of the transport of live fish have been discussed in detail.

The need has been pointed out for taking into account the size (especially the weight) effect in interpreting the data on the respiration of fish. While a mere grouping of fish in terms of their length-ranges or number in a given volume of water is not directly helpful in interpreting the experimental results, the factor, ml. (initial) oxygen per gm. fish, can be used with advantage to measure the influence of size on the respiration of the fish and to compare the viability of the fish under different environmental conditions.

On the basis of this factor, mortality of *Chanos* fry in hard tap water is seen to be relatively less than in sea-water; while, under conditions of asphyxiation, the median survival time of the fry does not appear to be affected to any significant extent by the salinity of the medium.

ACKNOWLEDGMENTS

Our grateful thanks are due to Dr. N. K. Panikkar for his interest in the work and to Sri. S. K. Banerji for his suggestions and advice in regard to the mathematical aspects of the paper. The collections of fish fry and fingerlings were made with the kind co-operation of Sri. D. A. S. Gnanadoss, Inspector of Fisheries (Madras), Pamban.

REFERENCES

Busnel, R. G., Drilhon, A. and Raffy, A. "Recherches sur la physiologie des Salmonides," *Bull. Inst. Oceanogr.* (Monaco), 1946, No. 893, 1–23.

Ganapathy, S. V., Chacko, P. I., Krishnamurthy, B. and Srinivasan, R. "On the acclimatisation, transport and culture of some salt-water fishes of the Madras State," *Ind. Geog. Jour.*, 1950, **25** (2), 1–15.

Keys, A. B. .. "A study of the selective action of decreased salinity and of asphyxiation on the Pacific killifish *Fundulus parvipinnis*," *Bull. Scripps Inst. Oceanogr.*, 1931, **2**, 417–90.

Le Cren, E. D. .. "The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*)," *J. Anim. Ecol.*, 1951, **20**, 201–19.

Paterson, D. D. .. *Statistical Technique in Agricultural Research*, New York and London, 1939, p. 113.

Rubner, M. .. "Aus dem Lebens des Kaltbluters. I. Teil: Die Fische," *Biochem. Zeitschr.*, 1924, **148**, 223–67.

APPENDIX I

(1) 100 *Chanos* fry, which had been in tap water for a day, were transferred to a limited volume, *viz.*, 3 l., of tap water (at 30° C.), kept in a tin fish carrier of effective capacity about 26 litres. The mortality of the fry 72 hours after the transfer was found to be nil. The mean length (l_1) of the fry was 20 mm. The dilutions and volumes for zero mortality in 72 hours for 100 fish have been calculated for various mean lengths of fish from equation (6) and are given below:

l_2 (mm.)	m	(mV/1000) l.
40	5·91	17·7
60	16·70	50·1
80	34·93	104·8
100	61·87	185·6

(2) 195 *Chanos* fry were transferred from a saline pond direct into 7·5 . of well water at 29° C. 96 of the fry died within 4 hours of transfer. The mean length of the fry was 20·5 mm. The calculations given below correspond to a total volume of well water of (7·5 × 3) l.

(i) For 50% mortality in 4 hours ($t_2 = t_1$),

l_2 (mm.)	$n_2 \times 3$
41	98
62	35
82	16
103	9

(ii) For 50% mortality, where $l_1 = l_2 = 20{\cdot}5$ mm., and $n_1 = 195 \times 3$,

$n_2 \times 3$	t_2 (hrs.)
1170	2
2340	1

(iii) For 50% mortality where $n_1 = n_2$,

l_2 (mm.)	t_2 (hrs.)
41	0·7
62	0·2

FUNGI IMPERFECTI FROM MADRAS—II

By C. V. Subramanian
(*University Botany Laboratory, Madras 5, India*)

Received August 11, 1952
(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

This is the second of a series of papers on the Fungi Imperfecti collected in the Madras State, India. All species mentioned except *Napicladium cratævæ* Syd. (which is now made the type of a new genus) are new records for India.

8. *Arthrinium saccharicola* Stevens apud Johnston and Stevenson in *J. Dept. Agric. Porto Rico*, 1917, **1**, 223. Saccardo, *Syll. Fung.*, 1931, **25**, 771.

Colonies black, up to 2·5 mm. in diameter. "Catkins" of conidia 40–105 × 4·8–6·4 μ. Conidiophores simple, erect or bent, closely aggregated, hyaline, with transverse septa occurring as dark bands at intervals of about 5–7 μ, 40–105 × 1·5–3 μ; conidia sessile, arising laterally from the cells of the conidiophore or from its apex, crowded together, pointed upwards simulating the appearance of a catkin, lenticular, pale brown in colour when mature, hyaline when young, 4·8–8·0 μ in diameter, 3·2–5·6 μ thick.

Only one collection has been examined: on dead stem, Thanthipandal, Kambakkam, Chingleput District, Madras State, 3–2–1952, coll. C. V. Subramanian (Herb. M.U.B.L. No. 718).

9. *Dictyoarthrinium quadratum* Hughes, in *Mycol. Pap. C.M.I.*, 1952, **48**, 30, ic.

Colonies black, irregular, powdery, up to 2 mm. broad, of variable length (up to 2 cm.), often coalescing. Mycelium composed of septate, branched, anastomosing, pale brown hyphæ, 2–5 μ broad. Conidiophores simple, densely crowded and aggregated, erect or oblique, straight or slightly curved or wavy, somewhat cylindrical, unbranched, subhyaline or pale brown in colour, up to 155 μ long, 3–5 μ broad, transversely septate, septa dark and simulating bands, cells of the conidiophore 3–10 μ long; conidiophores arising endogenously from somewhat cup-like, dark-coloured verrucose cells borne on short stalks or sessile and arising laterally on the hyphæ;

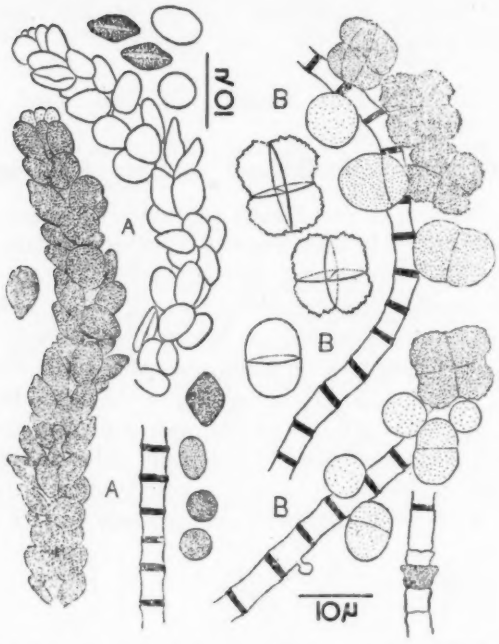


FIG. 1. A, *Arthrinium saccharicola* : conidiophores and conidia from Herb. M.U.B.L. No. 718; B, *Dictyoarthrinium quadratum* : conidiophores and conidia from Herb. M.U.B.L. No. 453.

verrucose cells 3–5 μ broad, 2–4 μ long; stalks 1–3-celled. Conidia when mature dark brown, thick-walled, verrucose, cruciately septate with four cells, somewhat squarish in outline and constricted at the septa, 11–13 μ in diameter, flattened, 8–10 μ thick, attached to the conidiophore apically or laterally; apical conidia sessile, borne singly at the apex of the conidiophore, in other respects similar to the lateral conidia; lateral conidia many, arising from cells of the conidiophore in single whorls and borne on short stalks, hyaline and spherical when young, echinulate and brown when mature.

Five collections have been made of which four are from the Travancore-Cochin State, South India: on dead leaves of *Cocos nucifera* L., Ernakulam (Travancore-Cochin State), 7–9–1951, coll. C. V. Subramanian (Herb. M.U.B.L. Nos. 453; 506); on dead leaf-sheath of *Musa paradisiaca* L., Ernakulam, 6–9–1951, coll. C. V. Subramanian (Herb. M.U.B.L. No. 464); on dead stem of (*Ixora* sp. ?) Rubiaceæ, Ernakulam, 7–9–1951, coll. C. V. Subramanian (Herb. M.U.B.L. No. 475); on dead grass culms (broom-

stick), University Botany Laboratory Garden, Madras, 20-12-1951, coll. C. V. Subramanian (Herb. M.U.B.L. No. 662).

10. *Podoconis theæ* (Bernard) Boedijn, in *Bull. Jard. bot. Buitenz.*, Ser. III, 1933, **13**, 133, ic.

Syn. *Helminthosporium theæ* Bernard. Saccardo, *Syll. Fung.*, 1913, **22**, 1390.

Mycelium pale brown to dark brown, branched, septate, 2–4 μ broad. Conidiophores brown, unbranched, 50–120 $\times$ 4–7 μ, up to 7-septate, the apical cell slightly swollen and narrowing to a tip 2–3 μ wide on which a single conidium is borne. Conidia acrogenous, borne singly at the tip of the conidiophore, 2–4- (mostly 3-) septate, somewhat obclavate, with a long narrowing apical cell and a short, narrow, appendage-like base, 50 $\times$ 9 (36–66 $\times$ 7–11) μ; the basal cell of the conidium brown in colour, and the broadest, the higher cells becoming progressively paler and narrower; the apical cell subhyaline, 14–26 $\times$ 2–3 μ. The conidiophores may proliferate through the scar of a fallen conidium and produce further conidia.

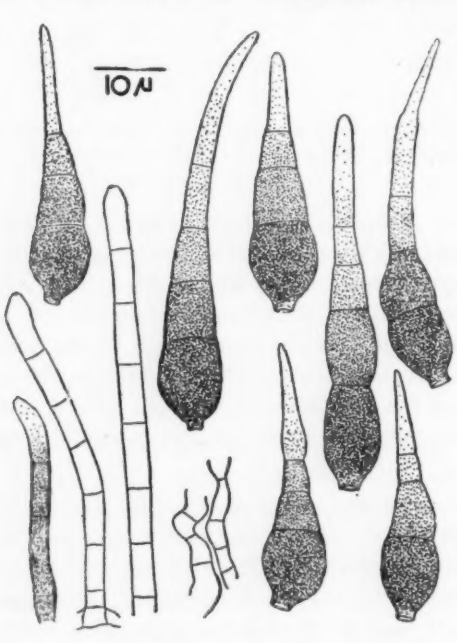


FIG. 2. *Podoconis theæ*: hyphæ, conidiophores and conidia from Herb. M.U.B.L. No. 539.

Only one collection has been examined: on dead leaf rachis of *Phœnix sylvestris* Roxb., Christian College Campus, Tambaram, near Madras (Chingleput District), 30-9-1951, coll. C. V. Subramanian (Herb. M.U.B.L. No. 539).

11. *Lacellina graminicola* (Berk. and Br.) Petch, in *Ann. roy. bot. Gdn., Peradeniya*, 1924, **9**, 171.

Syn. *Mesobotrys graminicola* (Berk. and Br.) Sacc. in *Syll. Fung.*, 1886, **4**, 325.

Colonies black, gregarious, velvetty due to the setæ, separate or coalescing, up to 2·5 mm. long. Conidiophores intermixed with setæ. Setæ simple, subulate, up to 940 μ long, 8–11 μ in diameter at the base, tapering upwards, black, becoming brown above and sometimes subhyaline to hyaline at the apex, with an obtuse apex, septate (up to 25 septa seen), the septa being distinctly visible only in the lighter coloured parts. Conidiophores erect or bent, straight or tortuous, simple, subhyaline to pale brown, septate, minutely verrucose throughout, up to 80 μ long, 3–5 μ broad.

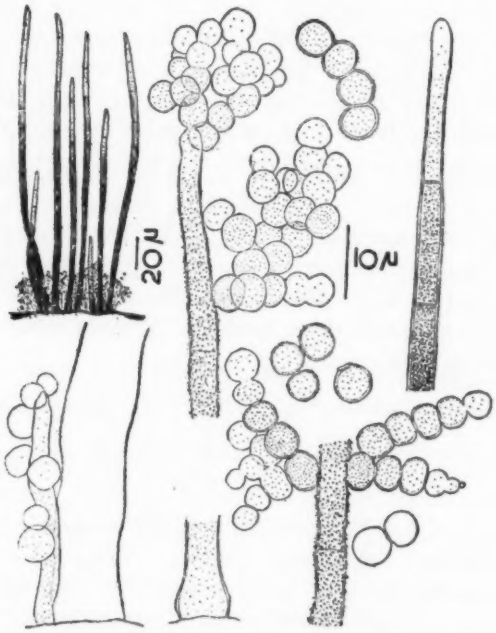


FIG. 3. *Lacellina graminicola* : setæ, conidiophores and conidia from Herb. M.U.B.L. No. 722.

Conidia acropleurogenous, one-celled, globose or oval, yellowish brown to dark brown, 4–7 μ in diameter (mean 5·6 μ), produced acropetally in branched or unbranched chains up to 56 μ long.

Two collections have been made: on dead stem of bamboo, Thanthipandal, Kambakkam (Chingleput District), 3–2–1952, coll. C. V. Subramanian (Herb. M.U.B.L. No. 722); on dead stem, Thanthipandal, Kambakkam, 3–2–1952, coll. C. V. Subramanian (Herb. M.U.B.L. No. 724).

12. *Napicladium cratævæ* Syd.

The type species of the genus *Napicladium* Thuem., *viz.*, *N. soraueri* Thuem. (*Hedwigia*, 1875, **14,** 4), has been shown to be synonymous with *Fusicladium dendriticum* (Wallr.) Fuckel by Winter (*Hedwigia*, 1875, **14,** 35–36). A new genus is therefore being proposed to accommodate Sydow's fungus.

Macræa Subramanian gen. nov.

Fungi imperfecti, phragmosporæ. Colonies effuse, brownish to black. Conidiophores simple, non-septate, erect or bent, straight or tortuous, subhyaline to brown, densely crowded. Conidia acrogenous, produced singly at the tip of the conidiophore, not in chains, napiform or obclavate, transversely 1-many-septate, broader at the base, tapering towards the tip, brownish towards the base, progressively paler towards the tip.

Pertinet ad Fungos imperfectos, atque ad Phragmosporas. Coloniæ effusæ, brunneolæ ad nigras. Conidiophori simplices, haud septati, erecti vel curvati, recti vel tortuosi, subhyalini ad brunneos, dense aggregati. Conidia acrogena, singula apici cuiusque conidiophori insidentia, haud catenulata, napiformia vel obclavata, transverse semel ad pluries septata, latiora ad basim, gradatim decrescentia ad apicem, brunneola ad basim, progressive pallidiora ad apicem.

Species typica: *Macræa cratævæ* (Syd.) Subramanian comb. nov.

Syn. *Napicladium cratævæ* Syd. in *Ann. mycol.*, Berl., 1913, **11,** 329. Saccardo, *Syll. Fung.*, 1931, **25,** 836.

Colonies rarely circular, more often irregular in shape, up to 5 mm. long, sometimes coalescing, somewhat velvety. Conidiophores simple, unbranched, non-septate, erect or bent, straight or tortuous, subhyaline to yellowish in colour, densely crowded, suddenly narrowed and somewhat conical towards the tip, 24–36 × 5·6–8·8 μ. Conidia acrogenous, produced singly at the tip of the conidiophore, not in chains, narrowly obclavate,

1–3-septate, the lower two cells brown in colour, the two apical cells paler in colour (almost subhyaline) and narrowing to an obtuse apex, 22–50×6·4–8·0 μ.

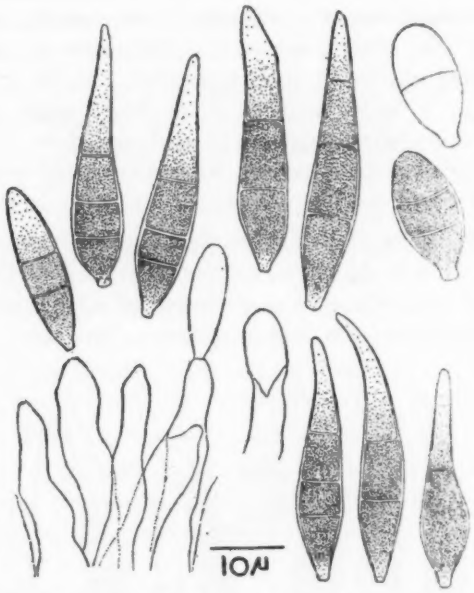


FIG. 4. *Macræa cratævæ*: conidiophores and conidia from type specimen *ex* Herb. Crypt. Ind. Orient. No. 2418.

The above description is based on a study of the type specimen of *Napicladium cratævæ* Syd.: on living leaves of *Cratæva religiosa* Forst., Government Farm, Coimbatore, Madras State, 5–2–1912, coll. W. McRae (No. 9) *ex* Herb. Crypt. Ind. Orient, No. 2418.

Through the courtesy of the Head of the Division of Mycology, Indian Agricultural Research Institute, New Delhi, I was able to examine part of a collection made by A. Khan " on living leaves of *Cratæva religiosa* Forst., from Karnal (E. Punjab), India, 13–10–1937 " and determined by him as *Napicladium cratævæ* Syd. This collection is in very good condition and showed an abundance of conidia. The colonies are darker in colour and larger than in the case of McRae's collection, the conidia are much longer and a majority of them are 4–6-septate. Further, the colonies are mostly circular in outline unlike McRae's fungus which has irregular spots. The fungus collected by A. Khan is therefore described here as a new species of the genus *Macræa*.

Macræa punjabensis Subramanian sp. nov.

Amphigenous, mostly hypophyllous. Colonies large, circular, rarely irregular, separate or coalescing, up to 1·5 cm. in diameter, brownish to deep black in colour, velvety. Conidiophores simple, unbranched, non-septate, erect or bent, straight or tortuous, yellowish to pale brown, densely crowded, suddenly narrowed and somewhat conical towards the tip, 24–40×6·4–7·2 μ. Conidia acrogenous, produced singly at the tip of the conidiophore, not in chains, napiform or obclavate, 1–6-, mostly 3–6-septate, the lower cells deep brown in colour, paler above, and with attenuate but obtuse apex, 27–64×6·4–7·2 μ.

Habitat.—On leaves of (?) *Cratæva religiosa* Forst., Karnal (E. Punjab), India, 13–10–1937, coll. A. Khan, *ex* Herb. Crypt. Ind. Orient. No. 12411 (Herb. M.U.B.L. No. 782). An examination of Khan's collection indicates that his determination of the host is probably incorrect.

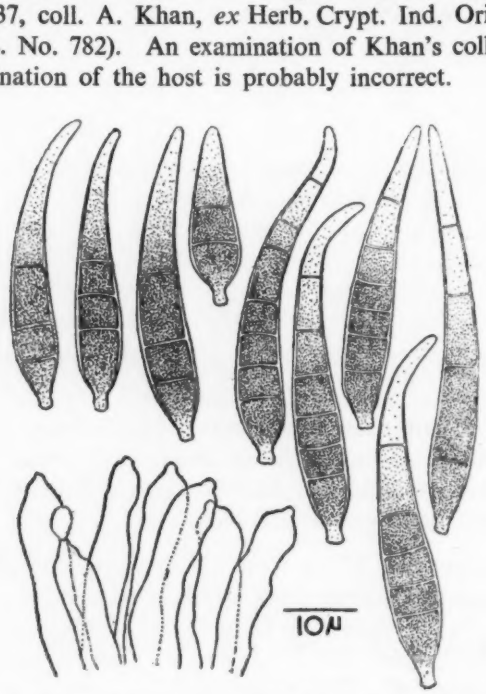


FIG. 5. *Macræa punjabensis* : conidiophores and conidia from type specimen *ex* Herb. Crypt. Ind. Orient. No. 12411.

Amphigena, ut plurimum hypophylla. Coloniæ magnæ, circulares, raro irregulares vel coalescentes, usque ad 1·5 cm. diam., brunneolæ ad profunde nigras colore, velutinæ. Conidiophori simplices, non-ramosi, non-septati, erecti vel curvati, recti vel tortuosi, luteoli ad pallide brunneos,

dense aggregati, subito angustati atque aliquantum conici ad apicem 24–40×6·4–7·2 μ. Conidia acrogena, singula apici cuiusque conidiophori insidentia, haud catenulata, napiformia vel obclavata, 1–6-, ut plurimum 3–6-septata; cellule inferiores alte brunneæ colore, superiores vero pallidiores, apice attenuato sed obtuso; 27–64×6·4–7·2 μ.

Habitat super folia (?) *Cratævæ religiosæ* Forst., Karnal (E. Punjab), India, 13–10–1937, coll. A. Khan; typus positus in Herb. Crypt. Ind. Orient, New Delhi, Sub. No. 12411 (Herb. M.U.B.L. No. 782).

13. *Camptomeris cratævæ* Subramanian sp. nov.

Colonies mostly hypophyllous, brown, irregular, up to 7 mm. long. Hyphæ branched, septate, subhyaline, 2–4 μ broad. Conidiophores simple, unbranched, one-septate near the base, cylindrical, yellowish to pale brown, straight or bent, erect, 24–32×5·6–6·4 μ. Conidia acrogenous, produced singly at the tip of the conidiophore, oblong to oblong-clavate with a papillate base, 1·6–2·4 μ broad, hyaline to subhyaline, up to 3-septate, with somewhat thick epispore, 19–34×4·8–6·4 μ.

Habitat.—On living leaves of *Cratæva religiosa* Forst., Agri-Horticultural Society's Gardens, Teynampet, Madras, 9–1–1952, coll. C. V. Subramanian and K. Ramakrishnan (Herb. M.U.B.L. No. 703).

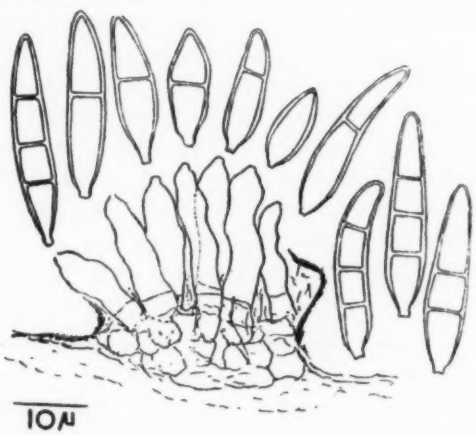


FIG. 6. *Camptomeris cratævæ*: conidiophores and conidia from type specimen *ex* Herb. M.U.B.L. No. 703.

Coloniæ ut plurimum hypophyllæ, brunneæ, irregulares, ad 7 mm. longæ. Hyphæ ramosæ, septatæ, subhyalinæ, 2–4 μ latæ. Conidiophori simplices, non-ramosi, semel septati prope basim, cylindrici, luteoli ad pallide brunneos,

recti vel curvati, erecti, 24–32×5·6–6·4 μ. Conidia acrogena, singula cuiusque conidiophori apici insidentia, oblonga vel oblonga-clavata, basi papillata, 1·6–2·4 μ lata, hyalina vel subhyalina, usque ter septata, episporio aliquantulum crasso, 19–32×4·8–6·4 μ.

Habitat in foliis viventibus *Cratævæ religiosæ* Forst.; typus lectus in hortis Agri-Hortic. Soc., Teynampet, Madras, die 9 januarii 1952 a C. V. Subramanian and K. Ramakrishnan, et positus in Herb. M.U.B.L. No. 703.

I thank Dr. R. S. Vasudeva, Head of the Division of Mycology, Indian Agricultural Research Institute, New Delhi, for lending me for study fragments from the type and other collections of *Napicladium cratævæ* Syd. in the *Herb. Crypt. Ind. Orient.* I am grateful to Professor H. Santapau of the St. Xavier's College, Bombay, for the Latin diagnoses.

A NEW SPECIES OF CŒLOMIC TREMATODE OF THE GENUS *STAPHYLORCHIS* FROM THE TIGER SHARK *GALEOCERDO TIGRINUS* FROM INDIAN WATERS*

By Mary Samuel

(*Central Marine Fisheries Research Station, Mandapam Camp*)

Received July 29, 1952

(Communicated by Dr. N. K. Panikkar, F.A.Sc.)

The trematodes of the sub-family Anaporrhutinæ Looss, comprise a number of genera, namely, *Anaporrhutum* Ofenheim, 1900, *Probolitrema* Looss, 1901, *Plesiochorus* Looss, 1901, *Petalodistomum* Johnston, 1913, *Staphylorchis* Travassos, 1920, and *Nagmia* Nagaty, 1930, which are known to infest the pericardial and cœlomic cavities of Sharks and Rays and the urinary bladder of marine turtles. In view of the extreme rarity of cœlomic trematodes and the fragmentary knowledge concerning those of Indian fishes, it seems proper to record certain observations on a new species of one of the largest cœlomic trematodes ever known. Possibly the earliest contribution on this group of trematodes in India is of Shipley and Hornell (1905) who recorded a number of specimens of *Distomum richiardii*, Lopez, from the cœlom of *Rhinodon typicus*. Later Max Luhe (1906) described the trematode *Anaporrhutum largum* collected by Professor Herdman from the cœlom of *Rhinoptera javanica*. Southwell (1913) recorded *Anaporrhutum largum*, Luhe from the body cavity of *Chiloscyllium indicum*, *Ginglymostoma concolor*, and *Aetobatis narinari* obtained from Ceylon and *Stegostoma tigrinum* caught off Orissa Coast and Chaudhuri (1916) whose work was based on parasites belonging to the same species *A. largum*, Luhe, added an additional host, *Hypolophus sephen* from the Chilka lake.

Nagaty (1930) has reviewed the sub-family Anaporrhutinæ and the genus *Staphylorchis*, supplementing previous descriptions of the known species *Anaporrhutum largum*, Luhe. A new Anaporrhutine trematode *Nagmia yorkei* was described by Nagaty who also gave a tabular description of all known genera of the sub-family Anaporrhutinæ. A critical review of the trematode parasites of elasmobranchs was published by

* Issued for publication with the kind permission of the Chief Research Officer, Central Marine Fisheries Research Station, Mandapam Camp.

Dolfus (1937). References concerning the genera may be obtained from the above papers which contain reviews of the earlier work.

Johnston (1913) created the genus *Petalodistomum* with *P. polycladum* as the type species and in it he included *P. cymatodes* from the cœlomic cavity of the leopard ray, *Dasybatus kuhlii* from Australian waters. Travassos (1920) formed a new genus *Staphylorchis* to accommodate *P. cymatodes* Johnston (1913). The new form described by Luhe (1906) as *Anaporrhutum largum* from the cœlomic cavity of the Ray *Rhinoptera javanica*, from Kalpitiya, Ceylon, has since been referred by Baylis (1927) to the genus *Staphylorchis*. Nagaty (1930) redescribed the specimens of *A. largum* from *Ginglymostoma concolor* from pearl banks, Ceylon and confirmed its position in *Staphylorchis* as indicated by Baylis.

GENERIC DIAGNOSIS OF *Staphylorchis* TRAVASSOS, 1920

The genus *Staphylorchis* clearly distinguishable by the large or above medium size, broad oval petal-like shape, smooth cuticle, and fleshy muscular body. Ventral and oral sucker more or less of the same size. Intestinal cæca sinuous and each diverticulum in the form of a continuous letter 'S' extending to the posterior margin of the body. Excretory pore median, sub-terminal and posterior. The genital pore median, preacetabular and behind the bifurcation of the gut. Testes 40 to 52 on each side of the body, spherical to oval, external to the intestinal crura. Ovary lobed or mulberry shaped, posterior to the acetabulum, right or left of the median line. Receptaculum seminis large, lateral to the ovary and post-acetabular. Vitellaria disposed in two lateral groups of branching follicles or tubules, post-acetabular and internal to the digestive cæca.

Staphylorchis gigas Spec. Nov.

Habitat.—Parasitic in the cœlom of *Galeocerdo tigrinus*.

Locality.—Madras and Nagapattinam, Bay of Bengal.

Diagnosis.—With the characters of the genus *Staphylorchis*. Large size, petal-like in shape with the posterior half of the body almost circular. Pharynx cup-shaped. Cirrus sac absent; vesicula seminalis comparatively long with the terminal portion dilated into a simple elongated oval sac. Ovary lobed, uterine coils filling space between the intestinal folds and may extend beyond terminal ends of the digestive cæca. Vitellaria in two compact group of branching finger-shaped follicles. Excretory vesicle tubular, elongate, reaching almost to the level of the posterior border of the ovary before bifurcation into lateral tubules.

Trematodes belonging to the genus *Staphylorchis* now recorded for the first time as occurring in Indian waters was found in the cœlomic cavity of the tiger shark, *Galeocerdo tigrinus* caught off Madras in May 1948, and Nagapattinam in June 1949. In the adult stage this species parasitizes the commercially important tiger shark. Of over a dozen specimens of tiger sharks examined, two harboured about a dozen of these parasites. These trematodes were found on opening the body cavity of the infested host and their resemblance to the description of *Petalodistomum cymatodes* (Johnston, 1913) indicated that the two were closely related. Further more, their morphological agreement with *P. cymatodes* suggested that they might belong to the same genus. The meagre account of the sexually mature worm of *P. cymatodes* given by Johnston is limited to the morphology of a single specimen and very little is known of the anatomical details. Since the specimens of the present study differ with the account of Johnston (1913) in certain specific characters as the general shape, greater size, structure of the male genital end organ, the shape of the ovary and vitelline follicles and the structure of the excretory system, and is also from a different host and different locality, their specific identity with those of Johnston's cannot be assumed.

The parasites were first expanded by spreading a few drops of rectified spirit over the surface of the body before fixing in 5% formalin. In sea water they are relatively sluggish and tend to remain in a contracted condition. The fixed specimens were dehydrated, stained in paracarmine and cleared in cedar wood oil for the study of internal anatomy. The measurements were taken from killed and formalin preserved material.

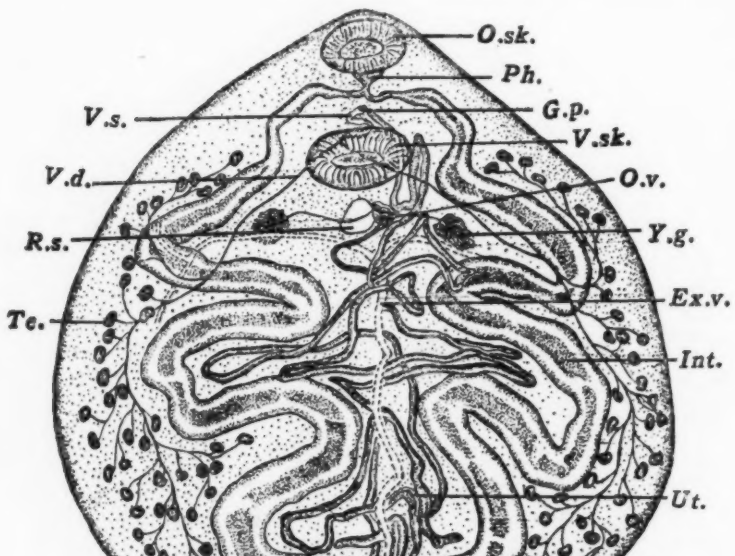
External Characters.—The parasites are large, chocolate brown in colour and conspicuous on the opened abdominal cavity of the host. They occur scattered throughout the length of the cœlomic cavity, although the majority are located on the surface of the liver; often they are deep between the lobes of the liver and may adhere tenaciously with the powerful acetabular sucker.

The shape of the body is portrayed in Photograph I and Fig. 1. The body is muscular, fleshy and smooth and does not bear any scales or spines. The cuticle is thick and smooth. In fixed specimens the contraction of the circular and longitudinal muscles gives the surface of the body at times, a ringed appearance. The shape of the body varies with the degree of extension. The worms are spherical to oval, the anterior extremity being produced into a conical projection while the posterior margin is broadly circular. The preacetabular region is more active and, when extended, it tends to bend ventrally forming a shallow ventral concavity.

V 36 1952 XUM

Gravid specimens measure from 25 mm. to 27 mm. in length and 20 mm. to 25 mm. in breadth. The oral sucker (*o. sk.*) is large, sub-terminal, spherical to ovoid in shape and with a longitudinal diameter of 1·8 mm. to 2 mm. and transverse diameter of 2·2 mm. to 2·6 mm. The acetabulum (*v. sk.*) lies within the anterior third of the body and measures from 1·8 mm. to 2·25 mm. by 2·25 mm. to 2·8 mm. in diameter. The apertures of the suckers are transverse and Johnston (1913) made no mention of the muscular development which determine the shape. The opening of the sucker is provided with a powerful spinchter which, when contracted, forms thickened muscular masses anterior and posterior to the apertures. These masses may protrude into the lumen giving the opening of the sucker a narrow slit-like shape.

General morphological features are shown in Fig. 1. A conspicuous feature of the worms is the two rami of the gut running on either side of the



TEXT-FIG. 1. *Staphylorchis gigas*, Sp. Nov. *Ex. v.*, excretory vessel; *Ex. p.*, excretory pore; *G.p.*, genital pore; *Int.*, intestinal limb; *O.sk.*, oral sucker; *Ov.*, ovary; *Ph.*, pharynx; *R.s.*, receptaculum seminis; *Te.*, testis; *Ut.*, uterus; *V.d.*, vas deferens; *V.s.*, vesicula seminalis; *V.sk.*, ventral sucker; *Y.g.*, yolk gland.

middle third of the body prominently as a pair of highly sinuous undulating tubes from the anterior end to the posterior end where they end blindly. The large number of testes occupying the whole length of the lateral margin of the worm external to the cæcum and the vitelline glands conspicuous as darkish aggregate of follicles lateral to the ovary and internal to the intestinal diverticulum are striking characters.

Digestive System.—The mouth opening is subterminal and is situated at the base of the oral sucker. A muscular pharynx (*Ph.*) leads directly out of the oral sucker and is followed by an œsophagus. The prepharynx is absent. The cup-shaped pharynx is 1 mm. anterio-posteriorly and 0·8 mm. transversely. The anterior half of the pharynx is hidden under the posterior lip of the oral sucker. The œsophagus is as long as or a little longer than the pharynx but the measurements could not be taken with certainty as the œsophagus was contracted in its longitudinal axis. The anterior portion of the œsophagus immediately behind the pharynx is narrow and tubular but the posterior end gradually widens and the intestinal cæca arise from the enlargement. The bifurcation of the digestive tract occurring anterior to the median genital pore is well in front of the ventral sucker. The intestinal diverticula (*Int.*) which are wide and unbranched as in *P. cymatodes* (Johnston, 1913) show a number of deep wavy undulations in the form of a continuous letter ' S ' and extend to the posterior border of the worm. The terminal portions curve inwards towards its fellow and then somewhat posteriorly and end blindly. There are ordinarily about three folds on either side, but this, however, may not be a constant number. The diameter of the cæcum ranges from 0·6 to 1 mm. The folds of the intestinal cæcum extend between the lateral border of the worm and more than half way to the mid-longitudinal axis and occupy a considerable part of the middle $\frac{2}{3}$ of the length and breadth of the fluke. The intestinal contents stain deeply and, as observed by Looss (1901), Ofenheim (1900) and Nagaty (1930) in other distomes, there is always a clear space between the wall and the contents.

Excretory System.—The excretory pore (*Ex. p.*) is median and subterminal and lies on the dorsal surface near the posterior end. It leads into a long narrow tubular vesicle (*Ex. v.*) which extends along the mid-longitudinal axis of the worm to almost the level of the posterior border of the ovary where it bifurcates into two undulating branches of narrower calibre. These branches pass obliquely towards the lateral regions of the body, reaching a position between the testicles and the intestinal folds where they divide again. In addition to the usual prolongation of the limbs towards the anterior ends of the body, there is a backward prolongation towards the posterior margins

of the body. The structure of the excretory system is noteworthy as it is different in its disposition from that of the type species *P. cymatodes*.

Reproductive System.—The large size of the worm is favourable for observing the reproductive system, the details of which can be completely studied in fixed and cleared specimens. The genital pore (*G.P.*) is median and lies on the ventral surface a short distance behind the bifurcation of the digestive tract and neither sinus nor papillæ are present. The male and female openings are close together, the male opening disposed slightly anterior to the female.

Male Genitalia.—The testes (*Te.*) consist of about 40 to 52 compact testicles on either side, varying in form from oval to spherical, oval ones averaging 0·8 mm. by 0·6 mm. while the spherical ones are 0·4 mm. to 0·8 mm. in diameter. They lie dispersed or in groups of two or three between the lateral margin of the body and the intestinal folds. The majority of them lies completely outside the intestinal diverticula but isolated testicles are to be found within the space bounded by the intestinal folds. The testicles extend anteriorly to the level of the acetabulum and posteriorly to the terminals of the intestinal ramus. Vasa efferentia arise from the mediar faces of the testicles and the ducts from the separate testicles join up in groups and converge towards the inner side of the area occupied by the testes and enter a longitudinal vas deferens (*V.d.*) on either side. The vasa deferentia cross the intestinal cæcum from the outer to the inner side ventrally, unite at the level of the anterior border of the acetabulum between the two intestinal cæca and pass forwards to a fairly long narrow slightly coiled vesicula seminalis (*V.S.*), the terminal portion of which dilates into a simple, elongated oval sac. The latter opens in the middle line approximately midway between the pharynx and the anterior border of the acetabulum and slightly anterior to the level of the female genital opening. This is different in structure from the vesicula seminalis as noted by Johnston (1913) in the type species *P. cymatodes* where he describes the vesicula seminalis as short. There is no cirrus or cirrus sac as described by Johnston (1913) for *P. cymatodes*. The testes are of the same type as in *P. cymatodes* but here they vary in shape from spherical to oval and at times lie in groups of two and three.

Female Genitalia.—The ovary (*Ov.*) is dorsal, situated immediately posterior to the acetabulum either median or lateral, right or left. It is slightly lobate, usually broader than long and in gravid specimens measures 1·7 mm. by 0·9 mm. The oviduct arises at the median side and the initial portion of the oviduct passes forwards into the ootype which receives the median vitelline duct and a duct from the receptaculum seminis and then

enlarges to form the uterus. The uterus (*Ut.*) passes backwards and is thrown into a series of close convolutions and the loops of the uterus follow a winding course which is irregular and difficult to follow. Unlike what has been described for *P. cymatodes*, the uterus is voluminous and fills the region between the intestinal diverticula and extends to the space bounded by the intestinal folds. The uterine coils reach almost to the posterior end of the body beyond the blind ends of the intestinal rami, return to the zone of the digestive cæca, pass backwards a second time to the region of the ovary and at the level of the vitelline duct run forwards as a more or less straight tube nearly to the level of the acetabulum and then folding on itself run back to the level of the vitelline gland and once again turn forwards forming a second loop before finally reaching the genital pore. In front of the ovary the uterus extends anteriorly as a moderately sinuous tube and joins the metraterm. The metraterm is almost straight and passes between the intestinal diverticulum and the terminal portion of the vesicula seminalis to the genital pore. The uterine coils are filled with numerous large oval-shaped, operculated eggs which measure 0·06 mm. by 0·03 mm.

The receptaculum seminis (*R.S.*) is spherical and prominent lying lateral to the ovary, slightly to the left or right of the middle line. It is larger than the ovary and its average diameter is 2·8 mm.

The vitellaria have the structure and disposition characteristic of the sub-family Anaporrhutinæ. The vitelline glands (*y.g.*) are paired and conspicuous and each gland consists of a cluster of branching follicles which resemble clumsy finger-like projections with rounded extremities. The vitellaria are ventral in position, post-acetabular and lateral to the ovary and receptaculum seminis and lie internally to the intestinal cæcum of its side. Their ducts have the usual disposition, a number of follicles unite to form a stem and a number of such stems unite forming the main transverse vitelline duct; the transverse ducts on either side unite between the ovary and the receptaculum seminis and join the oviduct on the right or left of the median line.

Discussion

The genus *Staphylorchis* was erected by Travassos (1920) to accommodate *Petalodistomum cymatodes* (Johnston, 1913) as the type and only known representative of the genus. Johnston (1913) described the adult stage of *Petalodistomum cymatodes* and distinguished between this species and *Petalodistomum polycladum*, the type of the new genus *Petalodistomum*. Travassos (1920) considered that *P. cymatodes*, although having many features in common with *P. polycladum*, could not be included in the genus

because of its possession of certain characters of generic value such as a highly sinuous but unbranched intestinal cæca and the structure, number and disposition of the testes.

The only other record of species of *Staphylorchis* is *S. largum* (Luhe, 1906; Baylis, 1927) from *Ginglymostoma concolor* by Nagaty (1930) but the generic identity of this species is greatly disputed. The species *Anaporrhutum largum* has been first described by Max Luhe (1906) after a specimen collected from the cœlomic cavity of *Rhinoptera javanica*, at Kalpitiya, Ceylon. Travassos (1922) in revising the family Gorgoderidæ, has included in the genus *Anaporrhutum*, Luhe's species *largum*. According to Baylis (1927) Luhe's species *largum* belongs to the genus *Staphylorchis* rather than to *Anaporrhutum*. Baylis's inclusion of this species in the genus *Staphylorchis* is based only on a single character of the position of the testes. Even the statement of Baylis that the testes in *Staphylorchis* " are situated entirely laterally to the intestinal branches " cannot be accepted since in *P. cymatodes* and the new species the testicles are distributed laterally between the level of the acetabulum and the posterior terminals of the cæcum and extend towards the median line, characters which do not appear in the species described by Luhe. The disposition and shape of the digestive cæca, the number and position of testes, disposition of the vitellaria and the position of the genital pore appear to be sufficient for including it in the genus *Anaporrhutum* as less difference exist between *Anaporrhutum* and Luhe's species *A. largum* than *Staphylorchis*. It is significant that the worms studied by Luhe (1906) and Nagaty (1930) came from the same locality, as Kalpitiya is very near Periya paar from where Nagaty obtained his specimens and in all probability Nagaty's species is identical with that of Luhe's.

In a critical review of the trematode parasites of Elasmobranchs Dollfus (1937) criticizes Baylis's allocation of *Anaporrhutum largum* Luhe, to the genus *Staphylorchis* and holds the view that *Anaporrhutum largum* is intermediate between *Anaporrhutum*, *Staphylorchis* and even *Petalodistomum* and as such it will be more justifiable to consider it as a type of a particular sub-genus than to attribute it to the genus *Staphylorchis*. Hence there is only one known species of *Staphylorchis* from which it remains to differentiate the species above described.

No difficulty arises in assigning the newly described species to its systematic position, the nature of the intestinal cæca and the number and disposition of the testes excluding all genera of the sub-family Anaporrhutunæ except one and this species falls naturally into the genus *Staphylorchis* Travassos. The new species in all probability is generically identical with

the type species *S. cymatodes* (Johnston, 1913; Travassos, 1920). It agrees with the type species in the sucker ratio, the form of the digestive cæca, the number and disposition of the testes. In addition it agrees in the large size of the receptaculum seminis and the position of the genital pore.

Although the species shows resemblance to *P. cymatodes*, a comparison of the present form with the description of the type as given by Johnston (1913) shows such definite differences that they cannot be included in the same species. They differ both in habitat and locality. The present material is collected from the cœlom of the tiger shark, *G. tigrinus* from the Indian coast. Johnston (1913) found the parasite in the body cavity of its host, the leopard ray, *Dasybatus kuhlii*, from Australian waters. The proposed new species *Staphylorchis gigas* differs substantially from *P. cymatodes* in the general shape and greater size. The size given for a mature specimen of *P. cymatodes* is 10·5 mm. in length and 8 mm. in breadth whereas the new species is more than twice as large as its congener measuring 25 mm. to 27 mm. in length and 20 mm. to 25 mm. in breadth. Differences, undoubtedly specific, which distinguished the species just described from *P. cymatodes* are to be found in the greater length of the vesicula seminalis, the structure of the male genital end organ and the absence of the cirrus sac. The new species differs from *P. cymatodes* in the shape of the vitellaria which are in the form of clumsy finger-like follicles in contrast to the " much branched tubes " of the type and the close proximity of the individual follicles of the new species gives to the vitellaria a rather compact appearance which is a specific character. In addition it differs from the type in the lobed shape of the ovary and the topographical relation of the uterine coils to the intestinal folds. A further important point of difference of specific significance is to be found in the excretory system, where the main excretory vesicle is long and tubular extending to below the level immediately posterior to the ovary before bifurcation to lateral branches, whereas in *P. cymatodes* the median excretory vesicle is very short and bifurcates at the level of the end of the digestive cæca in that figured by Johnston (1913). Any of these differences occurring separately may not have justified the erection of a separate species, but since a number of peculiarities occur in one species, it is considered that the specimens of *Staphylorchis* parasitic in the cœlom of Rays and sharks from Australian and Indian seas respectively are specifically distinct.

Summary

A new species of *Staphylorchis*, *S. gigas*, has been studied and its anatomical features are described. It is the only trematode parasite which has been found to infest the cœlomic cavity of the tiger shark, *Galeocerdo tigrinus*.

Sexually mature specimens are generically identical with the type species *S. cymatodes* (Johnston, 1913; Travassos, 1920) but since there are important specific differences such as the greater size, the form of the excretory system, shape of the ovary and vitellaria and the nature of the male genital end organ, they cannot be referred to the type species and hence described as a separate species. The discovery of this trematode from an Indian shark affords supplementary data concerning the little known genus *Staphylorchis*, previously found only in an Australian Ray and adds a second species to the genus which supports the validity of the genus.

ACKNOWLEDGMENT

I wish to thank Dr. N. K. Panikkar, Chief Research Officer, Central Marine Fisheries Research Station, Mandapam Camp, for going through the manuscript and suggesting improvements.

REFERENCES

Baylis, H. A. .. "Notes on three little known Trematodes," *Ann. Mag. Nat. Hist.*, 1927, **19** (9), 426–33, London.

Ben Dawes .. *The Trematoda. With Special Reference to British and Other European Forms*, Cambridge University Press, Cambridge, 1946.

Chaudhuri, B. L. .. "Fauna of the Chilka Lake. Fish. Pt. 1," *Memoirs of the Ind. Mus.*, 1916, **5**, No. 4, 403–39.

Dollfus, R. P. .. "Revues Critiques. Les Trematodes Digenea des Selaciens (Plagistomes) Catalogue Par Hotes. Distribution Geographique," *Annales de Parasitologie*, 1937, **15**, 57–73, 164–76, 259–81.

Johnston, S. J. .. "On some Queensland trematodes, with anatomical observations and descriptions of new species and genera," *Quart. Jour. Mic. Sci.*, 1913, **69** (1), Part 3, No. 235, N. ser. 361–400.

Looss, A.* .. "Ueber neue und bekannte Trematoden aus seeschildkroten," *Zool. Jahrb. Jena, Abt. F. Syst.*, 1901, **16**, 411.

Luhe, Max. .. "Report on the Trematode Parasites from Marine Fishes of Ceylon, Report to the Govt. of Ceylon on the Pearl Oyster Fisheries of the Gulf of Mannar", 1906, Part 5, 97–108.

Nagaty, H. F. .. "A new Anaporrhutine Trematode genus and species *Nagmia yorkei*, with a review of the classification of the sub-family," *Annals of Tropical Medicine & Parasitology*, 1930, **24**, 97–108.

Ofenheim, E Von .. *Ueber eine neue Distomiden gattung. Zeitschrift fur Naturwissen Schaften*, 1900, **73**, 1900–01.

Shipley, A. E. and Hornell, J. "Report on Pearl Oyster Fisheries," *Roy. Soc. Lond.*, 1905, **3**, 49.

Photograph 1

V36 1952 XUM

Southwell, T. .. "Notes from the Bengal Fisheries Laboratory, Indian Museum. On some Trematode and Cestode parasites from fish," *Rec. Ind. Mus. Calcutta*, 1913, **9**, 79–103.

Stunkard, H. W. .. "A new Trematode, *Probolitrema californiense* from the cœlom of the sting ray *Myliobatis californicus*," *Jour. Parasitology*, 1935, **21**, 359–64.

Travassos, L. .. "Contribuicœs para O Conhecinento da fauna helmintologica brasileira, XIV. Sobre as especies brasileiras da familia Gorgoderidæ Looss, 1901," *Brasil Medico, anno.*, 1920, **36**, 17–20.

———* .. "Contribuicoes para O conhecimento La fauna helmintologica brasileira, XVI. Gorgoderidae brazileiras," *Memorias do Instituto Oswaldo Cruz.*, 1922, **15**, 125-37, 220–34.

Woolcock, Violet .. "Digenetic Trematodes from some Australian Fishes," *Parasitology*, 1935, **27**, 309–31.

V36 1952 XUM

876-52 Printed at the Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent
and Published by The Indian Academy of Sciences, Bangalore

RELATIVE VALUE OF NITROGEN AND PHOSPHATE IN SOME ORGANIC MANURES AND INORGANIC FERTILISERS AND THEIR COMBINATIONS AS JUDGED BY THEIR RECOVERY BY CROPS

By S. V. Desai, F.A.Sc. and B. V. Subbiah
(Indian Agricultural Research Institute, New Delhi)

Received January 8, 1952

The field experiments conducted throughout India show that Indian soils respond most to nitrogenous manuring and that increased crop production mainly depends on the addition of nitrogenous manures and fertilisers. The form of nitrogen most suitable for different crops is an important aspect which has uptil now received only scanty attention in this country.

The relative crop-producing value of a fertiliser or manure varies greatly and is dependent on the type of fertiliser, the crop, soil and climatic conditions. Apart from the controversy on the relative effect of organic manures and inorganic fertilisers on the crop quality, their efficiency as judged by the nitrogen recoveries of the crop is also an important practical aspect. Although the fertilisers are coming into greater use, the main reason for the continued popularity of organic manures lies in their gradual availability of nitrogen to crops throughout the growing season. Moreover the nitrogen carried by them is not easily leached from the soil.

From a practical point of view, a suitable combination of organic and inorganic forms may be superior to either of them singly. Skinner (1931) of the United States Bureau of Chemistry and Soils reported that as a result of some 25 experiments with cotton on a variety of soil types and covering a period of four years, the highest yields were obtained when 80 to 85% of the nitrogen applied was derived from inorganic or synthetic sources and the rest from the proteid sources.

It is proposed to deal in this paper the results of field experiments conducted at the farm of the Indian Agricultural Research Institute, Delhi, on the comparative efficiency of different organic manures and inorganic fertilisers and their combinations, with respect to their nitrogen and phosphate.

Generally in many experiments, the relative value of nitrogenous fertilisers is based upon the increase in the crop yields that is produced by their

use. If the supply of nitrogen is more than adequate, in addition to increase in the yields, the percentage composition with respect to this nutrient increases. Thus the total recovery calculated by multiplying the yields and percentage composition, represents the total nitrogen made available by the fertiliser for the crop and hence will be a better criterion of the relative efficiency of fertilisers than their crop-producing power. As such, the nitrogen recovered by the crop is taken as the criterion for studying the relative efficiency.

EXPERIMENTAL

A field experiment was started in 1941 Kharif with the following treatments:—(1) Control, (2) F.Y.M. at 20 lb. N, (3) Activated sludge at 20 lb. N, (4) Rape cake at 20 lb. N, (5) F.Y.M. at 20 lb. N *plus* 20 lb. P_2O_5 *plus* 20 lb. K_2O, (6) Activated sludge at 20 lb. N *plus* 20 lb. P_2O_5 *plus* 20 lb. K_2O, (7) Rape Cake at 20 lb. N *plus* 20 lb. P_2O_5 *plus* 20 lb. K_2O, (8) Compost at 20 lb. N, (9) Compost at 10 lb. N *plus* 10 lb. N as ammonium sulphate *plus* 20 lb. P_2O_5, (10) F.Y.M. at 10 lb. N *plus* 10 lb. N as ammonium sulphate *plus* 20 lb. P_2O_5, (11) F.Y.M. at 60 lb. N and (12) Ammonium sulphate at 20 lb. N *plus* 20 lb. P_2O_5 and 20 lb. K_2O per acre. Phosphate and potash were added in the form of superphosphate and potassium sulphate in some of the treatments as shown above to study the effect of these two major elements on the nitrogen intake by the crops.

Lay out.—The experiment was laid out in randomised blocks with six replications and above 12 treatments in plots of 1/40th acre each in size. The crop rotation followed in the first four years was as follows:—1941, kharif—sorghum sown very thick for green fodder; 1941–42, Rabi—wheat; 1943, kharif—fallow; 1943–44, Rabi—wheat; 1944, kharif-fallow; 1944–45, Rabi—wheat. During the year 1944–45, the rate of application of manures was raised from 20 lb. to 40 lb. (and in the case of treatment 11, to 80 lb.) as 20 lb. N were considered too small. Manures were applied before every crop.

Soil.—The soil in this experimental field was a very light sandy loam, the mechanical composition of which shows about 80% sand, 10% silt and 10% clay up to a depth of 3 feet from the surface.

The relative efficiency of different fertilisers is presented below crop-wise.

Jowar Crop.—A fodder crop was grown in the kharif of 1941 in all the six replications and during 1942 in two blocks. The total nitrogen contents of the crop under various treatments are given below:

TABLE I. *Jowar Fodder*

Treatment (in lbs. per acre)	1941	1942	Average
	Average Nitrogen in lb. per acre of the crop		
1 Control ..	26·53	16·93	21·78
2 F.Y.M. at 20 lb. nitrogen ..	25·25	20·26	22·76
3 Act. sludge at 20 lb. nitrogen ..	28·00	33·44	30·72
4 Rape cake ,, ..	28·86	27·24	28·05
5 F.Y.M. ,, *plus* P *plus* K ..	27·06	20·74	24·20
6 Act. sludge ,, ,, ..	29·98	30·14	30·06
7 Rape cake ,, ,, ..	30·80	26·80	28·80
8 Compost at 20 lb. nitrogen ..	23·52	23·50	23·51
9 Compost at 10 lb nitrogen *plus* 10 lb. N as Amm. Sulph. 20 lbs. P_2O_5	29·64	23·36	26·50
10 F.Y.M. at 10 lb. *plus* rest as above ..	31·66	31·55	31·31
11 F.Y.M. at 60 lb. nitrogen ..	30·35	26·74	28·55
12 Amm. Sulph. 20 lb. nitrogen *plus* P *plus* K ..	30·67	30·78	30·73

Amongst the organic manures, F.Y.M. at 20 lb. N and 60 lb. N, Activated sludge, Rape cake and compost, Activated sludge proved to be the best as nitrogen carrier with 30·72 lb. of nitrogen taken up by the crop and are followed by Cake at 20 lb. N and F.Y.M. at 60 lb. N. Addition of phosphate and potash to these organics increased the nitrogen uptake both in the cases of F.Y.M. and rape cake and slightly depressed in the case of activated sludge. Combination of inorganic nitrogen with organic nitrogen (as F.Y.M. or compost) in equal proportions so as to make up 20 lb. N along with phosphate has increased the nitrogen uptake of the crop more than either of them singly. The complete artificial treatment was as good as the best organic manure which in the case of jowar appears to be activated sludge.

Maize Fodder.—The maize fodder was grown in two blocks during 1942 khariff and the recoveries of nitrogen under different treatments are given below in Table II.

TABLE II. *Nitrogen Recoveries by Maize Crop in lb. per acre*

Treatment (in lbs. per acre)	1942
1 Control ..	17·41
2 F.Y.M. at 20 lb. N ..	23·36
3 Activated sludge at 20 lb. N ..	26·85
4 Rape cake ,, ..	22·43
5 F.Y.M. at 20 lb. N. *plus* 20 lb. P_2O_5 *plus* 20 lb. K_2O ..	21·89
6 Activated sludge at 20 lb. N ,, ..	25·36
7 Rape cake ,, ,, ..	22·43
8 Compost at 20 lb. N ..	24·48
9 Compost 10 lb. N *plus* Amm. Sulph. 10 lb. N *plus* 20 lb. P_2O_5 ..	22·71
10 F.Y.M. at 10 lb. N *plus* same as above ..	18·92
11 F.Y.M. at 60 lb. N ..	27·08
12 Ammon. Sulph. at 20 lb. N *plus* P_2O_5 20 lb. *plus* K_2O at 20 lb. ..	28·20

Amongst the organic manures, F.Y.M. at 60 lb. N and activated sludge at 20 lb. N gave the highest nitrogen recoveries by the crop, 27·08 and 26·85 lb. of N respectively. Addition of phosphate and potash did not improve the nitrogen intake by the crop. Higher dose of F.Y.M. proved approximately equally as efficient as the treatment with complete artificials. Combination of organics with inorganics gave decreased nitrogen recoveries compared to either of them separately.

Wheat.—Wheat was the rabi crop grown during all the years of the experiment. During 1941–42 Rabi, wheat was grown after jowar fodder during 1942–43 rabi after maize and Jowar, and during 1943–44 and 1944–45 rabi after fallow. During 1944–45, the rate of nitrogen applied was doubled except in treatment with F.Y.M. at 60 lb. which was raised to 80 lb. N basis. The recoveries of nitrogen by wheat during each year are given in Table III below.

TABLE III. *Nitrogen in lb. per acre recovered by wheat crop*

	Treatment (in lbs. per acre)	1941–42	1942–43	1943–44	Average	1944–45*
1	Control ..	10·65	14·02	39·36	21·34	58·06
2	F.Y.M. at 20 lb. N ..	11·65	13·85	39·26	22·37	65·03
3	Act. sludge at 20 lb. N ..	14·84	18·12	40·13	24·36	65·16
4	Rape cake at 20 lb. N .	16·94	21·81	45·60	28·12	80·13
5	F.Y.M. 20 lb. N, 20 lb. P_2O_5, 20 lb. K_2O	15·35	15·70	44·60	25·22	60·63
6	Act. sludge 20 lb. N *plus* P & K as above	11·56	19·05	38·06	22·89	64·67
7	Rape cake at 20 lb. N *plus* P & K as above	15·20	20·37	42·46	26·01	66·07
8	Compost at 20 lb. N ..	14·30	14·09	35·81†	21·40	60·88
9	Compost *plus* Amm. Sulph. at 10 lb. N each *plus* 20 lb. P_2O_5	14·00	16·74	43·68	24·81	70·96
10	F.Y.M. *plus* the rest same as above	17·60	17·78	47·70	27·69	76·35
11	F.Y.M. at 60 lb. N ..	15·52	18·36	44·94	26·27	72·43
12	Amm. Sulph. at 20 lb. N *plus* P_2O_5 at 20 lb. and K_2O at 20 lb.	17·30	19·40	43·85	26·85	61·68

* During 1944–45 the rate of application has been raised from 20 lb. to 40 lb. except in treatment 11 where it was changed from 60 lb. to 80 lb.

† Excluding one replication of very low yields.

Considering the average figures for the first three years at a dose of 20 lb., amongst the organic manures, the highest recovery was by rape cake and is followed by F.Y.M. at higher dose (60 lb. N). Addition of inorganic P_2O_5 and K_2O depressed the nitrogen intake both in the cases of activated sludge and rape cake and slightly improved the nitrogen recovery in the case of F.Y.M. Combination of inorganic nitrogen half–half appears to be superior than single application of organic F.Y.M. or compost. Rape cake appeared to be superior practically all years over the treatment, complete artificials.

TABLE IV. *Amounts of Nitrogen taken up by crops on yearly basis per acre*

Treatment*	1941–42			1942–43				1943–44		1944–45	
	Kharif (jowar)	Rabi (wheat)	Total	Kharif (Maize–Jowar)	Average	Rabi wheat	Total	Kharif (Fallow)	Rabi (wheat)	Kharif (Fallow)	Rabi (wheat)
1 Control ..	26·53	10·65	37·18	(17·41 16·93)	17·17	14·02	31·19	..	39·36	..	58·06
2 F.Y.M. ..	25·25	11·65	36·90	(20·26 23·36)	21·81	13·85	35·66	..	39·26	..	65·03
3 Act. sludge .	28·00	14·84	42·84	(33·44 26·85)	30·15	18·12	48·27	..	40·13	..	65·16
4 Rape cake ..	28·86	16·94	45·80	(27·24 22·43)	24·84	21·81	46·65	..	45·60	..	80·13
5 '2'+P+K ..	27·06	15·35	42·41	(20·74 21·89)	21·32	15·70	37·02	..	44·60	..	60·63
6 '3'+P+K ..	29·98	11·56	41·54	(30·14 25·36)	27·75	19·05	46·80	..	38·06	..	64·67
7 '4'+P+K ..	30·80	15·20	46·00	(26·80 21·54)	24·17	20·37	44·54	..	42·46	..	66·97
8 Compost ..	23·52	14·30	37·82	(23·50 24·48)	23·99	14·09	38·08	..	35·81	..	60·88
9 Compost $\frac{1}{2}+\frac{1}{2}$N + P	29·64	14·00	43·64	(23·36 22·71)	23·04	16·74	39·78	..	43·68	..	70·96
10 F.Y.M. $\frac{1}{2}+\frac{1}{2}$N + P	31·06	17·60	48·66	(31·55 18·92)	25·24	17·78	43·02	..	47·77	..	76·35
11 F.Y.M. at 60 lb.	30·35	15·52	45·87	(26·76 27·08)	26·92	18·36	45·28	..	44·94	..	72·43
12 N+P+K ..	30·67	17·30	47·97	(30·78 28·20)	29·49	19·40	48·89	..	43·85	..	61·68

* Treatments same as in other tables.

Rotations and Nitrogen Recovery by Crops.—In the first two years, there was double cropping and later on only single crop was taken keeping the karif fallow. The total nitrogen taken up by crops on yearly basis is presented in Table IV.

It is seen from the above table that the nitrogen taken up by double cropping during 1941–42 and 1942–43 is approximately the same as by the single crop during 1943–44 with a fallow in kharif in spite of the fact that the nitrogen applied to both the crops amounts to double that applied to wheat in 1943–44. The significant increases in the nitrogen recovered by wheat during 1943–44 and 1944–45 appears to be accounted by probable gains in the nitrogen during the fallow preceding the wheat. During 1944–45 in addition to the previous fallow, the manures were applied at double the doses in the previous years and hence the nitrogen intake is considerably higher than the one obtained in the previous year.

Performance of Different Manures and Fertilisers year after year with Respect to Nitrogen intake by Wheat.—In order to evaluate the effect of continuous application of each treatment, the increase of nitrogen uptake by wheat over control by each treatment is given in Table V yearwise.

TABLE V. *Excess of Nitrogen absorbed by each Treatment over Control in lb. per acre*

Treatment (in lbs. per acre)	1941–42	1942–43	1943–44	1944–45*
1 Control ..	..	..	..	.
2 F.Y.M. at 20 lb. N ..	1·06	Nil	Nil	6·93
3 Act. sludge at 20 lb. N ..	4·19	4·10	0·77	7·10
4 Rape cake " ..	6·29	7·79	6·24	22·07
5 F.Y.M. at 20 lb. N *plus* P_2O_5 and K_2O at 20 lb. each	4·70	1·68	5·24	2·57
6 Act sludge *plus* the rest as above ..	0·91	5·03	Nil	6·61
7 Rape cake *plus* the rest as above ..	4·55	6·35	3·10	8·91
8 Compost at 20 lb. N ..	3·65	0·07	Nil	2·82
9 Compost at 10 lb. N *plus* Amm. Sulph. at 10 lb. N *plus* 20 lb. P_2O_5	3·35	2·72	4·32	12·90
10 F.Y.M. at 10 lb. N *plus* the rest as above	6·95	3·76	8·34	18·29
11 F.Y.M. at 60 lb. N ..	4·87	4·34	5·58	14·37
12 Amm. Sulph. at 20 lb. N *plus* P_2O_5 and K_2O at 20 lb. each	6·65	5·38	4·49	3·62

* During 1944–45, the rate of Application has been raised from 20 lb. to 40 lb. and in the treatment 11, from 60 lb. to 80 lb.

It is seen from this table that in the case of rape cake, the increase in the nitrogen uptake by the crop has been consistently maintained from year to year, while in the case of ammonium sulphate (treatment 12) it has progressively decreased from year to year in spite of addition of P_2O_5

and K_2O at 20 lb. rate and the increase in the rate of application of fertilisers during the years 1944–45. F.Y.M. at higher dose also gave consistently good increases over control throughout the period. The performance of activated sludge is not consistent. Compost and F.Y.M. at 20 lb. rate have generally failed to give any responses. Combination of organic manure like F.Y.M. or compost with ammonium sulphate each at 10 lb. N has substantially increased the nitrogen recovery from year to year.

Difference in performance of manures and fertilisers in Rabi and Kharif Seasons.—It is seen from Tables I and II that for kharif crops, activated sludge proved to be superior to rape cake generally while the reverse is the case for wheat in rabi. This difference in their performance is attributable to their rate of nitrifiability. In order to elucidate some of these points and to further study the relationship between nitrogen intake by the crops and the nitrifiability of the manures and fertilisers and their combinations, laboratory nitrification experiments with the same treatments were carried out with the same soil. The nitrogen was added at the rate of 30 mg. per 100 gm. of soil and in treatments containing P_2O_5 and K_2O, they were also added at the rate of 30 mgm. to hundred gm. of soil. The soils with these treatments were incubated at optimum moisture (12%) and optimum temperature (32° C.) which were found separately. The nitrate was determined periodically by phenol-disulphonic acid method at fortnightly intervals. The amounts of nitrate formed with time are given in Table VI. The figures represent average of duplicate determinations.

TABLE VI. *Nitrification of different Manures and Fertilisers*

(Nitrate in mgm. per 100 gm. of soil)

Treatment	Period in days						
	0	15	30	45	60	75	90
1 Control	0·53	3·1	3·3	3·0	3·0	3·2	3·45
2 F.Y.M. ..	1·30	4·8	5·4	4·3	5·1	5·1	4·8
3 Act. sludge ..	0·50	10·1	11·6	12·7	13·5	13·2	13·7
4 Rape cake ..	Trace	15·0	15·6	13·8	15·0	14·9	14·7
5 F.Y.M.+P+K ..	1·2	3·0	4·2	3·6	3·8	3·9	4·05
6 Act. sludge *plus* P & K ..	Trace	11·4	11·4	9·2	10·8	11·3	13·5
7 Cake *plus* P & K ..	„	14·4	15·6	14·4	13·2	14·4	16·8
8 Compost ..	3·45	7·4	7·8	7·4	7·2	7·4	7·8
9 Compost (½) Amm. Sulph. (½) *plus* P	1·8	16·5	17·6	13·2	12·8	14·4	17·7
10 F.Y.M. (½)+Amm. Sulph. (½) *plus* P	0·69	16·5	15·9	15·6	14·4	14·4	15·9
11 F.Y.M. at higher dose ..	..	..	..	..	..	..	..
12 Amm. Sulph.+P & K ..	0·6	21·3	28·4	27·0	24·0	24·0	23·5

Amongst the organic manures, F.Y.M., Compost, activated sludge and rape cake, the course of nitrification indicates that the maximum is almost reached after one month of incubation except in the case of activated sludge which reached the maximum later. Soon after the maximum was reached, there appeared to be fluctuation round about the steady value. The descending order of nitrification of these manures is as follows:—(i) cake, (ii) activated sludge, (iii) compost and (iv) F.Y.M. Although the conditions in the field are not optimum, these lab. nitrification studies generally indicate the extent to which the nitrogen in these manures would be available for crop growth. The easy nitrifiability of cake with chances of being leached out must have been responsible for its inferior performance during kharif as compared to activated sludge which nitrifies gradually. But because nitrogen in the cake nitrifies most, it has proved its superiority in Rabi with wheat.

The combined effect of phosphate and potash on the nitrification process appeared to slightly depress the course of nitrification, and this is also reflected in the nitrogen uptake by the crops. This depression is attributable to the addition of potash which is already present in Delhi soil in good available quantities and whose addition in general in this soil led to depressed yields. The results in Table VII reveal a negative correlation between the amounts of potash present in the organic manures and corresponding to 30 mgm. of total nitrogen and the amount nitrified.

TABLE VII

Manure	K_2O in 30 mg. of N in mg.	NO_3 formed (excess over control) in mg.
F.Y.M. ..	32·6	2·0
Compost ..	29·3	4·4
Act. sludge ..	10·3	10·25
Rape cake ..	6·7	12·15

Addition of inorganic nitrogen invariably increased the amount of nitrate formed. In the treatments 9 and 10 where the nitrogen is made up of half in the organic form and the other as ammonium sulphate, the amount of nitrate formed is additive taking the nitrifying value of ammonium sulphate and the manure separately as shown in Table VIII.

This position in more or less similar way is reflected in the nitrogen uptake by crops under these treatments.

TABLE VIII

		Nitrate formed over control
F.Y.M.	..	2·0
Ammonium sulphate	..	25·0
Compost	..	4·3
F.Y.M. (half) *plus* Amm. Sulph. (half) *plus* P—		
	Calculated ..	13·5
	Found ..	13·0
Compost (half) do. do.		
	Calculated ..	14·7
	Found ..	14·3

Nitrification in relation to total nitrogen intake by the Crop.—Nitrifiability of different manures is an important criterion of the nitrogen availability to the crop. It will be seen from Table X that manures and fertilisers which nitrify most have led to the maximum nitrogen intake by the crop.

Relative Value of Different Manures and Fertilisers on the Phosphate intake by the Wheat Crop.—In the following table are given the phosphate taken up by wheat crop under different treatments during 1942–43 and 1943–44 (average of six replications). The phosphate was determined by Fisky Subba Rao's method (Wright, 1939).

TABLE X. *Phosphate intake by Wheat Crop under Different Treatments*

Treatment	P_2O_5 in lb. per acre		Average	Excess over control
	1942–43	1943–44		
1 Control ..	7·46	13·66	10·56	..
2 F.Y.M. at 20 lb. N ..	8·49	13·66	11·08	0·52
3 Act. sludge ,, .	9·55	15·06	12·31	1·75
4 Rape cake ,, ..	12·24	16·44	14·34	3·78
5 F.Y.M. *plus* P_2O_5 and K_2O ..	9·33	16·92	13·13	2·57
6 Act. Sludge ,, ,, ..	10·66	15·12	12·89	2·33
7 Cake ,, ..	12·4	16·88	14·64	4·08
8 Compost at 20 lb. N ..	7·44	12·71*	10·08	Nil
9 Compost at 10 lb. *plus* Amm. Sulph. at 10 lb. *plus* 20 lb. P_2O_5	10·09	16·17	13·13	2·57
10 F.Y.M. *plus* the rest as above ..	10·84	18·71	14·78	4·22
11 F.Y.M. at 60 lb. N ..				
12 Amm. Sulph. at 20 lb. N *plus* P_2O_5 and K_2O at 20 lb. each	10·53	16·39*	13·46	2·90

* Excluding one plot of very low yield in the treatment.

It is seen from this table that amongst organic manures, there was highest intake of phosphate by the crop and addition of P_2O_5 and K_2O invariably increased the phosphate intake.

Addition of phosphate to a combination of inorganic and organic forms of nitrogen has also improved its intake by the crop.

SUMMARY

1. Taking the nitrogen recovery as the criterian of relative efficiency, amongst the organic manures F.Y.M., Compost, Activated sludge and rape cake, rape cake proved to be the best manure for Rabi (wheat) while activated sludge generally gave higher nitrogen recoveries for kharif crops like jowar and maize. F.Y.M. and compost at 20 lb. N were ineffective while F.Y.M. at 60 lb. was as good as rape cake at 20 lb. The phosphate recovery was also highest in the case of rape cake treatment.

2. Addition of phosphate and potash to the organic manures had generally a depressing effect both on the course of nitrification of the manures and on the nitrogen intake by crops (except for the first jowar crop). This depression may be due to excessive potassium, the availability of which is already high in the soil and any further additions appear to be bearing both. This combination has consistently enhanced the phosphate intake by wheat.

3. Combination of organic and inorganic nitrogen along with the phosphate gave good recoveries of nitrogen for all crops (except maize) and during all the years. Phosphate recovery was also good with this combination in the case of wheat crop.

4. Complete artificials comprising of nitrogen, phosphate and potash gave good nitrogen recoveries in the beginning of the experiment but year by year the nitrogen recovery decreased.

5. Generally manures which nitrified most also gave the best nitrogen recoveries.

ACKNOWLEDGEMENTS

Our thanks are due to Mr. C. H. Parr and Mr. R. D. Bose who were associated with the conduct of the field experiment on the farm.

REFERENCES

1. Skinner, J. J. .. *U. S. Dept. Agri. Tech. Bull.*, 1931, **225**, 1931.
2. Wright, C. H. .. *Agricultural Analyses* (excluding those for soils), 1939.

A CONTRIBUTION TO THE EMBRYOLOGY OF *CHLOROPHYTUM ATTENUATUM* BAK.

By K. Subramanyam and A. Nagaraja Rao
(*Department of Botany, Central College, Bangalore*)

Received September 8, 1952
(Communicated by Dr. L. Narayana Rao, F.A.SC.)

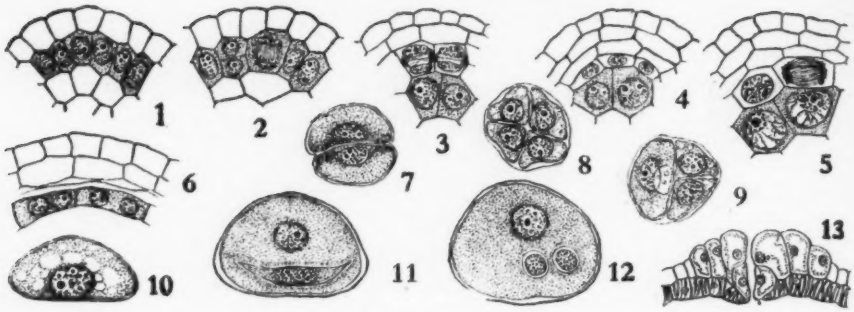
Introduction

The Liliaceæ is a very interesting family for it shows five distinct types of embryo sac development, *viz.*, Polygonum type, Allium type, Drusa type, Fritillaria type and Adoxa type. Eunus (1950) has drawn attention to this feature and an exhaustive review on these types of embryo sac development has recently been presented by Maheshwari (1948). The genus *Chlorophytum* is placed in the third sub-tribe Asphodeloideæ by Engler (1888) in the family Liliaceæ. In South India eight species of this genus are recorded (Gamble, 1925). *Chlorophytum attenuatum* is a perennial herb with cylindric, frequently tuber-like, roots. The inflorescence is a scape on which the white racemose flowers are arranged. The material was collected at Nandi Hills, a hill station about 35 miles from Bangalore, and fixed in formalin-acetic-alcohol. Sections were cut at a thickness of 14–18 microns and stained in Heidenhain's iron-alum hæmatoxylin with eosin as a counter stain.

Microsporangium and Male Gametophyte

The flower has six stamens arranged in two whorls of three each. A transverse section of the young anther lobe shows the hypodermal archesporium made up of a plate of six to eight cells (Fig. 1), with prominent nuclei and dense cell contents. They divide transversely forming the primary parietal layer and primary sporogenous layer (Fig. 2). The primary parieta layer by further divisions forms the wall of the anther (Figs. 3–6) and the sporogenous cells after undergoing a few more divisions become converted into spore mother cells (Figs. 3–5). The outermost layer in the young anther is the epidermis, next comes the endothecium, then a middle layer which soon becomes flattened and crushed (Figs. 4–6) and finally the glandular tapetum. The tapetal cells are uninucleate at first but later become binucleate (Figs. 5–6).

The microspore mother cells round up and undergo the usual reduction divisions. The first division is followed by the formation of a cell plate



FIGS. 1–13.

Fig. 1. Portion of cross-section of young anther showing archesporial cells, × 270. Fig. 2. Formation of primary parietal and primary sporogenous cells, × 270. Figs. 3 & 4. Stages in formation of anther wall, × 270. Fig. 5. Portion of young anther showing epidermis, endothecium, middle layer, uninucleate tapetum, and sporogenous cells; note division in one of the tapetal cells, × 270. Fig. 6. Same at a later stage showing crushed middle layer and binucleate tapetum, × 270. Fig. 7. Microspore Dyad, × 450. Fig. 8. Isobilateral tetrad of microspores, × 485. Fig. 9. Decussate opposite arrangement of microspores, × 485. Fig. 10. Uninucleate microspore, × 679. Fig. 11. Two-celled pollen grain, × 679. Fig. 12. Pollen grain showing tube nucleus and two male cells, × 679. Fig. 13. Portion of mature anther to show stomium and fibrous endothecium, × 120.

(Fig. 7); after the second division a cell plate is laid down in each of the dyad cells resulting in the formation of isobilateral tetrads (Fig. 8). Sometimes the microspores show a decussate arrangement (Fig. 9). A young microspore has a prominent nucleus surrounded by vacuolate cytoplasm (Fig. 10). Its wall is composed of a thin intine and a thick smooth exine with a single germinal furrow. The nucleus of the microspore divides to form an elongated generative cell and a rounded tube nucleus (Fig. 11). This is the shedding stage of the pollen grain, but occasionally the generative cell divides producing two male cells (Fig. 12).

In the mature anther, the tapetum and middle layer completely disorganise; only the epidermis and the endothecium persist and the latter develops characteristic fibrous thickenings. At the line of dehiscence some of the epidermal cells enlarge conspicuously and constitute the stomium (Fig. 13).

OVARY

The ovary is superior, tricarpellary, syncarpous and trilocular with two rows of axillary anatropous bitegmic ovules in each locule. The wall of the ovary consists of an uniform group of five to seven layers of thin-walled parenchymatous cells. In the mature fruit the cells of the innermost layer of the ovary wall develop lignified thickenings on their inner and tangential walls (Fig. 14). Prominent septal nectaries are present (Fig. 15).

Megasporangium and Female Gametophyte

Stages in the growth and curvature of the anatropous ovule are shown in Figs. 16–19. The micropyle is formed by the inner integument (Fig. 19) and it always grows earlier than the outer integument (Figs. 17, 18). The inner integument consists of two layers of cells except in the region of the micropyle where it is three to four layered. The outer integument consists of three to four layers of cells. The funicular strand ends at the base of the chalaza (Figs. 19, 20). To start with it consists of elongated cells (Fig. 20) which later show the characteristic thickenings of xylem (Fig. 21). At about the four-nucleate stage of the embryo sac, a biseriate row of nucellar cells at the chalaza connects the lower end of the embryo sac with the funicular strand. This row (Fig. 20) functions as a conducting strand for transporting food materials from the funicular strand to the antipodal end of the embryo sac.

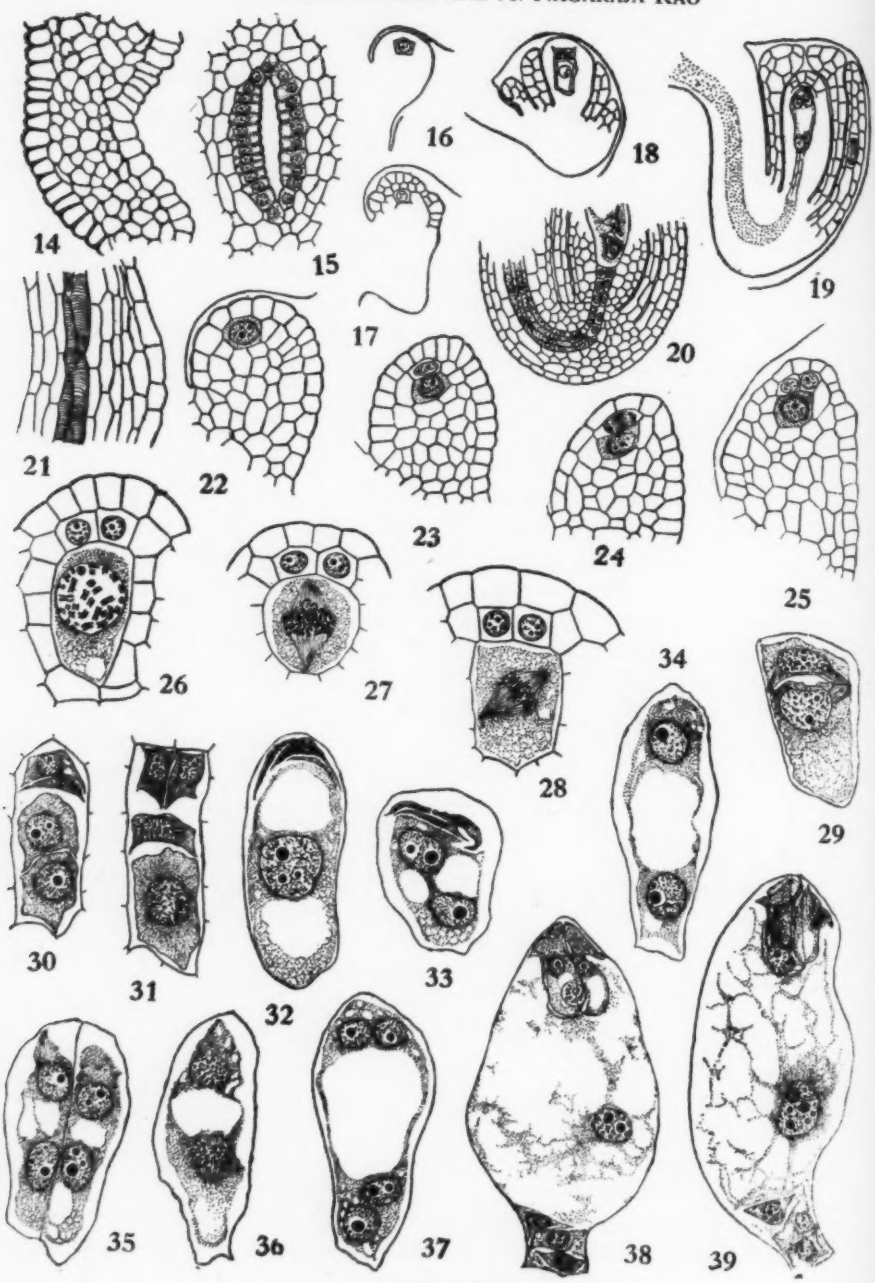
The hypodermal archesporium (Fig. 22) divides periclinally producing the parietal cell and the megaspore mother cell (Fig. 23). The parietal cell usually divides anticlinally (Fig. 24) producing two cells (Fig. 25). The megaspore mother cell soon enlarges in size (Fig. 26) and divides (Figs. 27) to produce the dyad. Sometimes the spindle is obliquely oriented (Fig. 28). Of the two cells of the dyad the upper is frequently smaller than the lower (Fig. 29). Both the cells divide again forming the linear tetrad (Fig. 30). A T-shaped tetrad is also met with (Fig. 31). The upper three megaspores degenerate and the chalazal megaspore (Fig. 32) undergoes three more divisions to produce the mature embryo sac which is of the Polygonum type (Figs. 33, 34, 36, 37 and 38). Fig. 35 shows two binucleate embryo sacs lying side by side.

The mature embryo sac (Fig. 38) is elongated and broad in the middle. Its chalazal end is in the form of a pouch. The synergids are hooked and sometimes exhibit a filiform apparatus. The pear-shaped egg is situated between them. The two polar nuclei fuse in the centre of the embryo sac to form the secondary nucleus which soon moves down. The antipodals are organised as cells and they lie in the chalazal pouch-like portion of the embryo sac.

The entry of the pollen tube is porogamous and double fertilisation occurs (Fig. 39).

Endosperm

The primary endosperm nucleus divides near the lower end of the embryo sac by a transverse or slightly oblique wall to form two chambers


14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39

FIGS. 14–39

Fig. 14. Portion of mature ovary wall, × 30. Fig. 15. Section of septal nectary × 60. Figs. 16–19. Stages in development of anatropous ovule, × 120 × 129; × 129; × 129. Fig. 20. Chalazal portion of ovule enlarged showing conducting strand, × 129. Fig. 21. A portion of funiculus enlarged showing thickening in funicular strand, × 296. Fig. 22. L.S. young nucellus showing primary archesporial cell, × 270. Fig. 23. Primary parietal cell and megaspore mother cell, × 270. Fig. 24. Anticlinal division in primary parietal cell, × 270. Fig. 25. Two parietal cells and megaspore mother cell, × 270. Fig. 26. Megaspore mother cell in diakinesis, × 485. Fig. 27. Megaspore mother cell in metaphase, × 450. Fig. 28. Oblique division in megaspore mother cell, × 485. Fig. 29. Formation of dyad, × 485. Fig. 30. Linear tetrad, × 485. Fig. 31. T-shaped tetrad, × 485. Fig. 32. Uninucleate embryo sac, × 450. Fig. 33. Formation of two-nucleate embryo sac, × 450. Fig. 34. Two nucleate embryo sac, × 450. Fig. 35. Two binucleate embryo sacs lying side by side, × 450. Fig. 36. Formation of four-nucleate embryo sac, × 450. Fig. 37. Four-nucleate embryo sac, × 450. Fig. 38. Mature embryo sac showing synergids with filiform apparatus, egg, secondary nucleus and antipodal cells, × 679. Fig. 39. Double fertilisation, × 301.

of which the micropylar is larger than the chalazal (Fig. 40). The nuclei in both these chambers divide again (Fig. 41). At this stage the cytoplasm in the lower chamber is very dense and later it completely disorganises. Free nuclear divisions occur rapidly in the micropylar chamber (Fig. 42) and the nuclei become parietally placed by the formation of a central vacuole. After wall formation sets in, further growth is centripetal and a massive cellular endosperm is produced. A Helobial type of endosperm has also been reported in other species of *Chlorophytum* (Stenar, 1949). In the mature seed its cells become densely filled with starch grains (Fig. 57).

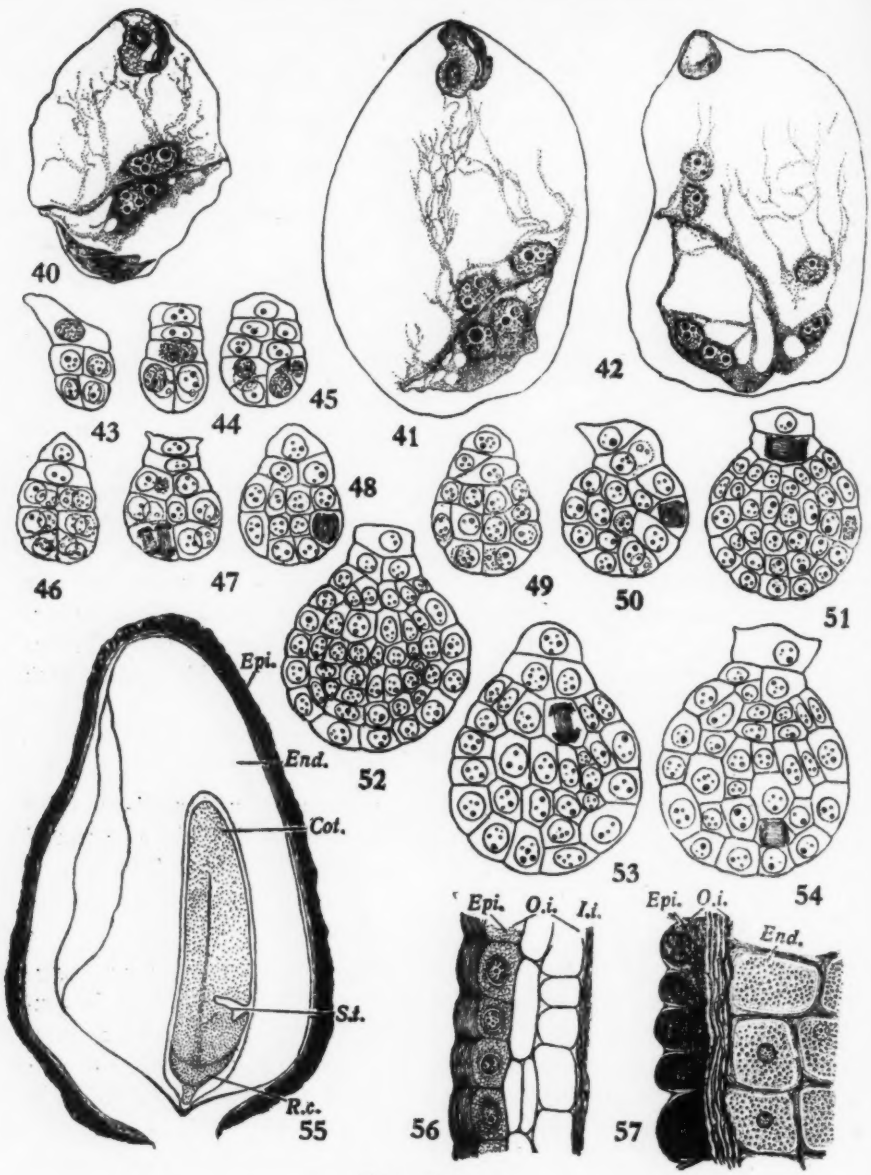
Embryo

Stages in the development of the embryo are shown in Figs. 43–55. The mature embryo is elongated and monocotyledonous, with a terminal cotyledon, lateral stem tip and the root covered by a root cap (Fig. 55).

Seed Coat

During the development of the seed, the inner integument gets completely crushed. The external wall of the epidermal layer of the outer integument gets gradually thickened (Fig. 56), while the inner layers become more or less compressed by the developing endosperm. Finally only the epidermal layer persists; its cells become filled with dense contents which take up a deep stain (Figs. 55, 57).

Our sincere thanks are due to Prof. P. Maheshwari for valuable suggestions and Prof. L. N. Rao for kind encouragement.



FIGS. 40–57.

Figs. 40–42. Early stages in development of endosperm according to Helobia. type, × 291; × 291; × 215. Figs. 43–54. Stages in development of embryo, × 291; Fig. 55. L.S. mature seed, × 30. *Cot.*, cotyledon; *End.*, Endosperm; *Epi.*, Epidermis. *R.c.*, Root cap; *S.t.*, Stem tip. Figs. 56, 57. Stages in development of seed coat, × 270; × 450. *End.*, Endosperm; *Epi.*, Epidermis; *I.i.*, Inner integument; *O.i.*, Outer integument

SUMMARY

The wall of the young anther is made up of four layers of cells. The tapetal cells are binucleate and are of the glandular type. A stomium is organised at the line of dehiscence. The microspores are arranged in an isobilateral manner. The mature pollen grain is two-celled.

Septal nectaries are present on the ovary wall. The ovules are anatropous and bitegmic, and the micropyle is formed by the inner integument. A conducting strand consisting of a biseriate row of cells is seen at the chalazal end of the ovule and this connects the base of the embryo sac with the funicular strand.

The development of the embryo sac follows the Polygonum type. Double fertilisation is observed.

The endosperm is Helobial and its cells are full of starch.

The mature embryo is monocotyledonous. Only epidermal layer of the outer integument persists in the mature seed.

LITERATURE CITED

Engler, A. and Prantl, K. .. *Die Natürlichen pflanzen familien*, 1888, **2** (4), 32–35, Leipzig.

Eunus, A. M. .. "Contributions to the embryology of the Liliaceae, 1," *J. Indian Bot. Soc.*, 1950, **29**, 68–78.

Gamble, J. S. .. *The Flora of the Presidency of Madras*, 1925, London.

Maheshwari, P. .. "The Angiosperm Embryo Sac," *Bot. Rev.*, 1948, **16**, 1–56.

Stenar, H. .. "Zur Kenntnis der Embryologie und der Raphiden-zellen bei *Bowiea volubilis* Harvey und anderen Liliazeen," *Acta Horti Bergiani*, 1949, **15**, 45–63.

STUDIES IN THE PHYSIOLOGY OF PHANEROGAMIC PARASITISM WITH SPECIAL REFERENCE TO *STRIGA LUTEA* LOUR. AND *S. DENSIFLORA* BENTH. ON *ANDROPOGON SORGHUM* HACK.

II. The Influence of Temperature and Moisture on the Germination and Growth of *S. lutea and S. densiflora*

By S. Solomon

(Botany Section, College of Agriculture, Poona)

Received August 17, 1951

[Communicated by Prof. L. S. S. Kumar, M.Sc. (Lond.), F.A.Sc.]

Introduction

In the first article in this series (Solomon, 1952), studies on the osmotic pressure of the host and parasite in relation to the nutrition of the host were reported. In the present article, the influence of temperature and moisture on the germination and growth of the parasites will be discussed.

It has been reported by Pearson (1913) and confirmed by Saunders (1933) that *S. lutea* seeds do not germinate in the absence of the hosts roots. The latter investigator has shown that contact with the host roots is not absolutely necessary for germination, which takes place if *S. lutea* seeds are watered by the drainage water obtained after irrigating the host plants. Saunders (1933) did some experiments in order to determine the nature of the substance excreted from the host roots which is necessary for initiating the germination of *S. lutea* seeds and came to the conclusion that it is either a proteolytic enzyme or an amide. Brown and Edwards (1944) have reported that the rate of germination of *S. lutea* seeds, after exposure to the host stimulus, is enhanced by previous exposure of the seeds to moist, warm conditions. This is taken to indicate that the host factor only operates at a comparatively late stage in the germination process. Andrews (1945) reports that the greater majority of the seeds of *S. hermonthica* can only be germinated by excretions from roots of certain plants not all of which can act as hosts for this parasite. Brown and Edwards (1945) have very recently succeeded in substituting thiourea and allylthiourea for the host stimulus. By treating the seeds with a 0·1 to 1% solution of these chemicals for 1–6 days and incubating at 34° C. considerable germination was obtained. Vallance (1950, 1951) has reported some studies on the germination of

Striga hermonthica and he suggests that the specific metabolite required for germination might be proteinaceous. On the other hand, Todd as quoted by Russell (1949) considers the stimulating substance excreted by the roots to be a sugar-*d*-xyloketose.

Since *S. lutea* seeds need not be in contact with the host roots in order to germinate, Saunders (1933) was able to determine the minimum, optimum and maximum temperatures for the germination of the parasite seeds by watering them with the excess water obtained after percolation through a large pot of sand containing young maize seedlings. In his experiments the optimum conditions for the germination of *S. lutea* seed were afforded by a temperature of 30° to 35° C., while no germination took place at 15° C. or 45° C.

Material and Methods

In the course of these studies, the effect of temperature on the germination of *S. lutea* and *S. densiflora* seed has been tested in the presence of a growing *Sorghum* seedling, as it was felt that any separation of the host and parasite would be unnatural. This has set a limit to the temperatures that could be used, as it was found that *Sorghum* seeds germinated very poorly at 40° C. It was also observed that the room temperature varied between 20° C. and 30° C. during the period of the experiment and hence a temperature below 30° C. could not be used. Therefore, in the case of *S. lutea* seed only two temperatures were tried, *viz.*, 30° C. and 35° C. with a control at room temperatures, *i.e.*, 20° C. to 30° C. For *S. densiflora* seed, the same temperatures, *viz.*, 30° and 35° C. were used but the control varied between 25° to 30° C. The germination tests were carried out in petri dishes in which a pinch of *Striga* seed had been sprinkled. One seed of a highly susceptible variety of the host was placed in each dish and the dishes were placed in incubators. The dishes were removed at the end of a fortnight and the number of *Striga* seeds in the dish and the number that had germinated was counted under a lens. From these figures the percentage germination in each dish was calculated.

These results have been tested statistically and the differences between the different treatments and the control have been found to be significant. These results indicate that 30° C. is the most favourable temperature for the germination of *S. lutea* seeds. In Saunder's (1933) experiments too 30° C. gave the highest percentage germination. As regards *S. densiflora* seeds, the results show that 35° C. is the optimum temperature for their germination. At this temperature the percentage germination is five times that in the control and twice that at 30° C. It is noteworthy that the optimum

TABLE I

Effect of Temperature on the Germination of S. lutea *and* S. densiflora *Seeds*

Species	No. of dishes tested	Temperature	Percentage germination per dish	
			Range	Mean
S. lutea	30	Control: 20°–30° C.	0–67	23·5
,, ...	30	30° C.	2–88	46·9
,, ...	30	35° C.	0–44	14·1
S. densiflora ..	27	Control: 25°–30° C.	0–14	2·8
,, ..	27	30° C.	0–40	7·5
,, ..	27	35° C.	0–35	14·4

temperature for the germination of *S. lutea* seeds, *i.e.*, 30° C., is not the optimum for *S. densiflora* seeds which require the higher temperature of 35° C. Moreover, *S. densiflora* seeds do not germinate as readily as those of *S. lutea* do. Even at the optimum temperature of 35° C., *S. densiflora* seeds show a germination of 14·4% as against that of *S. lutea* at 30° C. of 46·9%.

THE INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE ON THE GROWTH AND ATTACKING ABILITY OF *S. lutea* AND *S. densiflora*

Several workers have pointed out the importance of soil temperature as a factor influencing the distribution or severity of soil-infesting fungi. Among the diseases in which soil temperature has been shown to play a leading role, the following may be mentioned: Take all disease of wheat (Davis, 1925, and McKinney, 1925); pea blight and root-rot (Jones, 1923), cabbage yellows (Gilman, 1916, Tisdale, 1923; and Tims, 1926); *Gibberella saubinetti* (Dickson, 1923), flax wilt (Tisdale, 1917), and tobacco root-rot (Johnson and Hartmann, 1919).

It has been pointed out by Jones and his associates (1926) that one should not lose sight of the importance of other environmental factors. For example, temperature may be only one of the influential variables with a disease like common scab of the potato where the influence of soil moisture or of soil reaction may also be highly important. With still other diseases as club root of cabbage, it has been shown that temperature may have only

a minor influence, while other factors such as soil reaction or soil moisture may be dominant.

As the main object of the *Striga* scheme financed by the Indian Council of Agricultural Research was to build up strains of *Sorghum* resistant to *Striga* attack, it was decided to determine the optimum temperature and moisture conducive to maximum attack and subject the strains selected for resistance, to a high degree of infestation for a prolonged period under the optimum conditions of attack for selecting immune types.

Experiments on S. lutea

Two experiments were done *to find out the optimum temperature and moisture at which maximum attack of* S. lutea *occurs.*

The first experiment was started in January 1940, in the newly constructed glass-house which was equipped with ten soil temperature tanks, capable of holding eight cans each. The cans were placed in water which was warmed by electric heaters. Temperature was controlled by means of thermostats. Moisture content of the soil was regulated by placing a known weight of soil in a weighed can and adding water daily to bring it to a predetermined weight. Combinations of three temperatures, *viz.*, 30° C., 35° C. and control at soil temperatures varying between 20° and 30° C., and three moisture levels, *viz.*, 20%, 30% and 40% on basis of oven-dry soil, were tried. There were eight replications for each treatment. River soil was used. It had a maximum water-holding capacity of 50% and the moisture in air dry soil was 7% by weight. All the cans were sown on 6th January 1940 with seed of a susceptible variety of *Sorghum* and infected with 0·2 gm. of *S. lutea* seed. While sowing, three seeds of the host were placed in each can. On 29th January, *i.e.*, after three weeks, the extra host plants were removed, only one *Sorghum* plant being left in each can. During this operation, it was observed that some of the plants had already been attacked by *Striga*. During February 1940, the growth of the host plants was very rapid in all tanks except those in which the moisture content was maintained at 40%. This moisture content was very near the maximum water-holding capacity of the soil and hence was not suitable for the growth of the *Sorghum* host (*vide* Plates X & XI). In February also, *Striga* plants began to appear above the surface of the soil. The date of appearance of the first *Striga* plant in each can was recorded. Weekly observations were made on the number and height of the tallest *Striga* plant in each can. The data obtained for the number of *Striga* plants and the height of the tallest *Striga* plant sixty days from the date of sowing are tabulated below. It will be observed that only in two treatments, *viz.*, 35° C. and 20% moisture and 35° C. and 30%

moisture were all the host plants attacked. As the experiment was performed out of season, it was not possible to determine the effect of these treatments on the growth and yield of the host.

Results of the First Experiment

TABLE II

Number of S. lutea *Plants at Different Temperatures and Moisture Levels*

Treatments		Cans (Blocks)								Total	Mean
Temp. °C.	Moisture %	1	2	3	4	5	6	7	8		
30	20	0	0	4	1	1	0	2	0	8	1·00
30	30	0	0	0	4	0	3	0	3	10	1·25
30	40	0	0	0	3	0	0	0	0	3	0·37
35	20	13	14	7	17	4	14	10	16	95	11·87
35	30	10	7	8	4	5	8	4	3	49	6·12
35	40	1	1	0	0	0	0	1	1	4	0·50
Uncontrolled	20	0	0	0	1	0	0	0	0	1	0·12
„	30	0	1	0	0	0	0	0	0	1	0·12
„	40	0	0	0	0	0	0	0	0	0	0

TABLE III

Height in Cm. of the Tallest Striga lutea *Plant in Each Can at Different Temperatures and Moisture Levels*

Treatments		Cans (Blocks)								Total	Mean
Temp. °C.	Moisture %	1	2	3	4	5	6	7	8		
30	20	..	..	14	2·5	2·5	7·5	..	..	26·5	3·3
30	30	..	2·5	..	5	7·5	7·5	..	5	27·5	3·4
30	40	..	..	..	2·5	..	..	..	..	2·5	0·3
35	20	9	47·5	15	25	42·5	17·5	20	19	195·5	24·4
35	30	15	11	9	17·5	12·5	9	17·5	11	102·5	12·8
35	40	2·5	1	..	..	..	..	1	1	5·5	0·7
Uncontrolled	20	..	..	..	6	..	..	..	..	6	0·7
„	30	..	1	..	..	..	..	..	..	1	0·1
„	40	..	..	..	..	..	..	..	..	..	0

From the statistical analysis of the results obtained in this experiment, the conclusion seemed to be justified that 35° C. and 20% moisture level was capable of inducing the greatest attack of the host and ensuring the best growth of *S. lutea*. In order to confirm these findings, a second experiment was started in June 1940. The technique followed was similar to the one recorded in the previous experiment. The same soil (river soil) was used. The temperatures and moisture levels tried were slightly different from the previous ones, *viz.*, combinations of four temperatures 32° C., 35° C., 38° C., and uncontrolled soil temperatures varying between 20° and 40° C., and three moisture levels, *viz.*, 20%, 25% and 30% were used. One seed of a susceptible variety of *Sorghum* was sown in each can and alternate cans were infected with 0·2 gm. of *S. lutea* seed, the object being to study the effect of attack on the yield of straw and grain of the host plants. As the experiment was performed in the proper season for growing *Sorghum*, it should have also been possible to determine the optimum temperature and moisture for the growth of the host under the conditions of the experiment. Unfortunately this could not be done as the host plants were severely attacked by mites and aphis after a month from sowing and no remedial measures could mitigate the effects of this attack. The experiment had, therefore, to be concluded prematurely. The number of *S. lutea* plants in each can was recorded once a week. *Striga* plants were first observed in less than a month from sowing in almost all constant temperature series and even the cans placed on the glass-house bench at air temperatures showed attack in 40 days. This was contrary to the results obtained in the first experiment where only two treatments, *viz.*, 35° C. and 20% moisture, and 35° C. and 30% moisture showed uniform attack in all the cans. The data obtained for the number of *S. lutea* plants in each can and the height of the tallest *Striga* plant 50 days after the date of sowing are tabulated below.

From the results obtained, it was observed that the temperature treatments have given highly significant differences in both the experiments. It can, therefore, be concluded that 35° C. is the optimum temperature for the growth of *S. lutea*. However, in the second experiment, the moisture treatment and the interaction between temperature and moisture are not significant. It was found that it was not possible to maintain the levels of moisture, *viz.*, 20%, 25% and 30% with any degree of accuracy by the method adopted in this experiment. This might explain the diversity in results between this experiment and the first one where a definite significance for the moisture treatments 20%, 30% and 40% and their interaction with temperature has been recorded. It was, therefore, decided to perform

Results of the Second Experiment

TABLE IV

Number of S. lutea *plants at Different Temperatures and Moisture Levels*

Treatments		Cans (Blocks)				Total	Mean
Temp. °C.	Moisture %	1	2	3	4		
32	20	4	11	8	4	27	6·75
32	25	5	15	14	6	40	10·00
32	30	14	21	16	3	54	13·50
35	20	16	14	10	12	52	13·00
35	25	16	10	16	14	56	14·00
35	30	7	9	17	10	43	10·75
38	20	8	11	10	14	43	10·75
38	25	13	10	4	4	31	7·75
38	30	14	4	6	4	28	7·00
Uncontrolled	20	4	Nil	7	3	14	3·50
„	25	9	1	Nil	3	13	3·25
„	30	2	2	2	Nil	6	1·50

TABLE V

Height in Cm. of the Tallest S. lutea *Plant in each Can at Different Temperatures and Moisture Levels*

Treatments		Cans (Blocks)				Total	Mean
Temp. °C.	Moisture %	1	2	3	4		
32	20	1	15	11	12	39	9·75
32	25	12	17	7	15	51	12·75
32	30	12	12	7	1	32	8·00
35	20	12	16	13	11	52	13·00
35	25	21	15	15	18	69	17·15
35	30	15	10	12	17	54	13·50
38	20	6	6	7	6	25	6·15
38	25	6	6	7	4	22	5·50
38	30	12	7	12	1	32	8·00
Uncontrolled	20	2	Nil	2	2	6	1·50
„	25	2	1	Nil	1	4	1·00
„	30	12	1	1	Nil	14	3·50

separate experiments to determine the optimum moisture level for the attack and growth of *S. lutea* using a technique which guaranteed a better control of the moisture level.

Moisture content of the soil required for the maximum attack and growth of S. lutea.—The technique employed in these experiments for maintaining the desired moisture level was a modification of that used by Bailey (1940). Moisture content was regulated by placing a known weight of soil in a weighed can and adding water to bring it to a predetermined weight. In order that the amount of water added may be evenly distributed throughout the soil in the can, three glass tubes reaching to different depths were inserted and water equally distributed between them. The tubes rested on layers of sand so as to allow the water to spread evenly. The surface of the soil in the can was also covered with a thin layer of sand in order to prevent evaporation as much as possible. Three levels of moisture were tried, *viz.*, 20%, 25% and 30% (on basis of oven-dry soil). River soil was used. There were 8 cans for each treatment making 24 cans in all. Seeds of a susceptible variety of *Sorghum* were sown in all the cans, the soil of which was infected with 0·2 gm. of *S. lutea* seed, in August 1942. The cans were placed on the bench in the glass-house. The treatments were randomised. The date of first appearance of *Striga* in each can was recorded. The *Striga* plants were seen about 40 days after starting the experiment and within 60 days of sowing all the host plants were attacked.

In these experiments, two criteria were adopted for determining the optimum moisture required for the maximum attack and growth of *S. lutea*, *viz.*, (1) the number of *Striga* plants appearing in each can, and (2) the total height attained by these *Striga* plants. For this purpose, the number of *S. lutea* plants in each can were counted and their height measured once a week. The highest figures that were obtained in these counts are tabulated below and used for statistical analysis. After the experiment was over, the moisture in the cans was tested and found to approximate very closely to that being maintained.

Further, it was observed that no *Striga* plants appeared in can No. 4, and only one each in can Nos. 5 and 6 of the treatment for 30% moisture. The host plants in these cans were unfortunately attacked by stem-borer and their growth was very poor. The *Sorghum* plant in can No. 4 died at the end of September and though those in can Nos. 5 and 6 survived till the end of the experiment, their growth was very poor. The single *Striga* plants that appeared in these cans died very soon after their appearance, *i.e.*, long before flowering. It was, therefore, apparent that the results obtained in

can Nos. 4, 5 and 6 of the treatment for 30% moisture were abnormal and could not be used in the statistical calculations. These values were, therefore, termed *a*, *b* and *c*, and fresh values were calculated for them by the method used by Yates (1933). The calculated values have been placed below the letters *a*, *b* and *c*. As the values for the maximum number of *Striga* plants are small whole numbers, the square roots of these values were employed for calculating the analysis of variance as recommended by Cochran (1938). The degrees of freedom for error were reduced by the number of calculated values.

TABLE VI

Number of S. lutea *Plants at Different Moisture Levels*

Treatments	Cans (Blocks)								Total	Mean
	1	2	3	4	5	6	7	8		
20% moisture ..	19	17	11	4	3	5	4	3	66	8·25
25% moisture ..	11	12	20	15	19	8	19	13	117	14·62
30% moisture ..	21	17	26	*a* 18	*b* 19	*c* 14	21	19	155	19·38

TABLE VII

Total Height in Cm. of S. lutea *Plants in each Can at Different Moisture Levels*

Treatments	Cans (Blocks)								Total	Mean
	1	2	3	4	5	6	7	8		
20% moisture ..	79	78	42	36	9	25	34	34	337	42·1
25% moisture ..	98	101	151	115	146	63	145	95	914	114·2
30% moisture ..	202	190	236	*a* 179	*b* 183	*c* 149	95	224	1458	182·2

The experiment was repeated in order to obtain confirmation. The technique employed in this experiment for maintaining the desired moisture level and other details of procedure were the same as in the previous experiment, except that there were six replications instead of eight. The experiment was started in August and concluded in December 1943. The data

for the maximum number of *S. lutea* plants in each can and the total height attained by them are tabulated below and subjected to statistical analysis.

TABLE VIII

Number of S. lutea *Plants at Different Moisture Levels*

Treatments	Cans (Blocks)						Total	Mean
	1	2	3	4	5	6		
20% moisture ..	5	5	6	2	3	6	27	4·50
25% moisture ..	5	4	5	2	5	7	28	4·66
30% moisture ..	6	7	9	3	9	13	47	7·83

TABLE IX

Total Height in Cm. of S. lutea *Plants in each Can at Different Moisture Levels*

Treatments	Cans (Blocks)						Total	Mean
	1	2	3	4	5	6		
20% moisture ..	43	25	26	6	15	42	157	26·2
25% moisture ..	79	84	98	17	63	122	463	77·2
30% moisture ..	70	121	97	54	106	102	550	91·7

From the results of both the experiments it may be confidently concluded that 30% is the optimum moisture level for the attack and growth of *S. lutea*.

Experiments with S. densiflora

Optimum temperature for maximum attack and growth of S. densiflora.—In view of the experience gained while performing the experiments for determining the optimum temperature and moisture for the attack of *S. lutea*, it was decided to study the effect of each of these factors separately for *S. densiflora*. The experiment was started in June 1942 and concluded in September 1942. The following treatments were tried: 30° C., 35° C. and glass-house temperatures varying between 20° to 40° C. There were 8 cans under each treatment. Temperature was controlled in the constant

temperature series in the manner described in the first experiment. The cans exposed to fluctuating temperatures were placed on the glass house bench. Other details were similar to that described in the previous experiment. Seed of a susceptible *Sorghum* variety was sown in all cans, the soil of which was infected with 0·2 gm. of *S. densiflora* seed. The number of *Striga* plants in each can was recorded as they came up. Their heights were also measured once a week. *S. densiflora* plants were first observed a month after sowing and in two months all the host plants were attacked. The majority of *S. densiflora* flowered about 90 days from sowing when the experiment was concluded. In some cases the first *S. densiflora* plants that came up died soon after and others appeared later. This happened most often in the constant temperature series, *viz.*, 30° C. and 35° C. In the series exposed to the air temperatures on the bench, the *S. densiflora* plants invariably flowered in about a month after making their appearance.

TABLE X

Number of S. densiflora *Plants in each Can at Different Temperatures*

Treatments	Cans (Blocks)								Total	Mean
	1	2	3	4	5	6	7	8		
30° C. ..	1	8	3	1	5	3	3	3	27	3·37
35° C. ..	4	5	2	4	1	7	14	3	40	5·00
Uncontrolled (20°–40° C.)	12	5	8	7	7	6	7	1	53	6·62

The differences for the number of *S. densiflora* plants attacking a single host in the different temperatures tried are not significant.

TABLE XI

Total Height in Cm. of the S. densiflora *Plants in each Can at Different Temperatures*

Treatments	Cans (Blocks)								Total	Mean
	1	2	3	4	5	6	7	8		
30° C. ..	1	129	8	1	71	41	26	3	280	35·0
35° C. ..	24	40	15	50	1	175	346	10	643	80·4
Uncontrolled (20°–40° C.)	275	204	242	204	147	123	204	8	1407	175·9

The heights attained by plants in the cans placed at fluctuating temperatures are significantly greater than those at constant temperatures. The differences between 35° C. and 30° C. are not significant.

The experiment *to determine the optimum temperature for the maximum attack and growth of* S. densiflora, was repeated in 1943. The same treatments, *viz.*, 30° C., 35° C. and uncontrolled temperatures between 20° and 40° C. were tried. The experiment was started in June 1943 and concluded in October 1943. There were eight cans for each treatment. The data for the maximum number of *S. densiflora* plants and the total heights attained by them are tabulated below.

TABLE XII

Number of S. densiflora *Plants in each Can at Different Temperatures*

Treatments	Cans (Blocks)								Total	Mean
	1	2	3	4	5	6	7	8		
30° C. ..	8	0	1	3	5	0	3	4	24	3·0
35° C. ..	2	5	2	6	5	6	2	4	32	4·0
Uncontrolled (20°–40° C.)	11	5	3	6	5	11	2	3	46	5·7

As in the previous experiment, the number of *S. densiflora* plants appearing in a single can are not statistically different in the three treatments tried.

TABLE XIII

Total Height in Cm. of the S. densiflora *Plants in each Can at Different Temperatures*

Treatments	Cans (Blocks)								Total	Mean
	1	2	3	4	5	6	7	8		
30° C. ..	185	0	42	88	139	0	126	112	692	86·5
35° C. ..	70	155	77	105	157	118	46	119	847	105·
Uncontrolled (20°–40° C.)	258	132	151	189	164	179	109	115	1297	162

As in the previous experiment, the results show that *S. densiflora* plants in the cans placed on the glass-house bench make the best growth. The differences between 35° C. and 30° C. are not significant.

The results obtained in both these experiments are similar. It may be concluded that the number of *S. densiflora* plants appearing in each can, is not significantly different for the three treatments tried. But *S. densiflora* plants in the cans kept on the glass-house bench at temperatures ranging between 20° and 40° C. grow much faster and attain much bigger heights than at constant temperatures of 35° C. and 30° C. Judged by this criterion, therefore, temperatures ranging between 20° to 40° C., every 24 hours, are the optimum for the growth of *S. densiflora*.

It has previously been shown that *S. densiflora* seeds germinate best at 35° C., though, even so, the germination is very poor, not exceeding 14·5% at 35° C. It is, therefore, not surprising to find the number of *S. densiflora* plants appearing under different treatments are statistically the same. However, it must be noted that there is one optimum for the germination of *S. densiflora* seeds and another for the growth of *S. densiflora* plants.

Optimum moisture level required for the maximum attack and growth of S. densiflora.—The procedure adopted in this experiment for maintaining the desired moisture level in the soil was similar to that used in the third and fourth experiment. The experiment was commenced in August 1943 and concluded in December 1943. The following moisture levels were tried: 20%, 25% and 30% on weight of oven-dry soil. There were 6 cans for each treatment which were randomised on the concrete bench of the glass-house. The seeds of a susceptible variety of *Sorghum* were sown in all cans, the soil of which was infected with 0·2 gm. of *S. densiflora* seed. The number of *S. densiflora* plants in each can and their total height was recorded once a week. The maximum values obtained in these were used for computation.

TABLE XIV

Number of S. densiflora *Plants in each Can at Different Moisture Levels*

Treatments	Cans (Blocks)						Total	Mean
	1	2	3	4	5	6		
20% moisture ..	0	0	0	2	1	0	3	0·50
25% moisture ..	0	2	1	4	5	0	12	2·00
30% moisture ..	3	1	3	2	4	3	16	2·67

TABLE XV

Total Height in Cm. of S. densiflora *Plants in each Can at Different Moisture Levels*

Treatments	Cans (Blocks)						Total	Mean
	1	2	3	4	5	6		
20% moisture ..	0	0	0	23	9	0	32	5·3
25% moisture ..	0	34	27	98	83	0	246	41·0
30% moisture ..	92	18	52	46	74	106	388	64·7

Both from the point of view of number of *S. densiflora* plants attacking a single host and the total height attained by them, the results of this experiment are inconclusive. It however appears clear that 20% is not the optimum moisture level for the maximum attack and growth of *S. densiflora*. The differences between 30% and 25% are not significant.

DISCUSSION AND CONCLUSIONS

Of the seven experiments reported here, four have been performed with *S. lutea* as the parasite and three with *S. densiflora*. It would, therefore, be of interest to note here our findings on the optimum temperature and moisture level required for the maximum attack and growth of these two species of *Striga*.

In the first place, constant temperatures of 35° C. are the best for inducing the largest number of *S. lutea* plants to attack a single host and for the *S. lutea* plants to make the best growth in height. As regards *S. densiflora*, it was found that none of the three temperatures tried, *viz.*, 35° C., 30° C. and uncontrolled (20°–40° C.) showed any significant differences in the number of *S. densiflora* plants attacking a single host but at temperatures ranging from 20°–40° C. in 24 hours, *i.e.*, the control on the glass-house bench, the *S. densiflora* plants were more vigorous and grew much taller.

Secondly, 30% moisture level was the optimum for the maximum attack and growth of *S. lutea*; the same moisture level gave the best result for *S. densiflora* also but the differences between it and 25% were not statistically significant.

For the same treatments, a much larger number of *S. lutea* plants appeared than of *S. densiflora*, but *S. densiflora* plants were much taller than those of *S. lutea*.

As 35° C. is found to be the optimum temperature for inducing the maximum attack and growth of *S. lutea* and temperatures ranging between 20° C. and 40° C. for *S. densiflora*, the question arises whether these temperatures do occur under field conditions and, if so, during what season and how long they prevail. In order to ascertain this, soil temperature readings were taken at 9 A.M., 3 P.M. and 6 P.M. (S.T.) at 3″ and 6″ depth in the field. The mean values are plotted in the form of a graph in Fig. 1. From the data presented it appears that the necessary conditions required for inducing

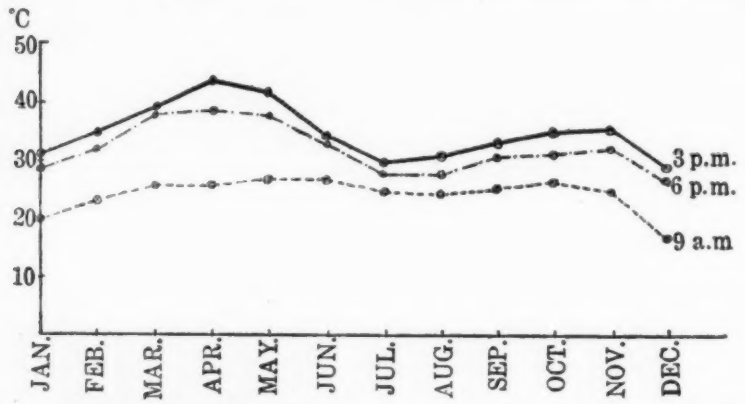


FIG. 1. Average soil temperatures throughout the year at 9 A.M. 3 P.M. & 6 P.M. (S.T.)

the attack and growth of *S. lutea* and *S. densiflora* are present from March to November every year.

As regards the moisture content of the soil during the growing season of the *Sorghum* crop, this would vary from year to year according to the rainfall and its distribution. *Striga* occurs in a severe form in all *Sorghum* growing areas, the rainfall of which may vary from 15″ to 35″ per year. The attack is most virulent and the greatest damage is done when the rainfall is less than normal. It has been observed that if rainfall is continuous during the first two months of the crop's growth, *Striga* appears, if at all, in a very mild form. Further it may be mentioned that frequent irrigation checks the attack of *S. lutea* and to some extent that of *S. densiflora* also. Andrews (1945) has also found that light irrigation of the *Sorghum* crop during the normal sowing period increases the attack of *S. hermonthica* and heavy irrigation decreases it. But if the intervals are longer than a week or 10 days, the ameliorative effect is lost. From this it would appear that *Striga* abhors excessive moisture. This has been confirmed by our preliminary studies in which the attack of *S. lutea* was almost nil at 40% moisture level. On

the other hand, from the fact that 30% moisture level is the optimum both for *S. lutea* and *S. densiflora*, it may be concluded that a certain amount of moisture is necessary for the growth of *Striga* and that it is not a xerophytic plant.

Summary

1. The relation of temperature to the germination of *S. lutea* and *S. densiflora* seeds was determined. The temperatures tried were 30° C., 35° C. and a control at room temperatures which ranged between 20° and 30° C. The tests were carried out in petri-dishes containing one seed of a susceptible variety of *Sorghum* along with those of the parasite.

2. 30° C. was found to be the optimum for *S. lutea* and 35° C. for *S. densiflora*. Room temperatures were the next best for *S. lutea* and 30° C. for *S. densiflora*. 35° C. gave the lowest germination for *S. lutea* and room temperatures for *S. densiflora*. The germination of *S. densiflora* at 35° C. was only 14·4% as against 46·9% of *S. lutea* at 30° C. (its optimum).

3. The optimum temperatures for the maximum attack and growth of *S. lutea* and *S. densiflora* have been determined by growing the parasites along with the hosts in cans placed in soil temperature tanks and on the glass-house bench.

4. The optimum moisture level of the soil for the attack and growth of *S. lutea* and *S. densiflora* have been determined by growing the parasites along with the hosts in cans placed on the glass-house bench. The desired moisture level was maintained by a modification of the method adopted by Bailey (1940).

5. Constant temperatures of 35° C. induce the largest number of *S. lutea* plants to attack a single host and the *S. lutea* plants make the fastest growth in height.

6. None of the three temperatures tried, *viz.*, 35° C., 30° C. and uncontrolled (20°–40° C.) showed any significant differences in the number of *S. densiflora* plants but in the last treatment the *S. densiflora* plants made the best growth.

7. 30% Moisture level was the optimum for the maximum attack and growth of *S. lutea*.

8. 30% moisture level also gave the best results for *S. densiflora* but the differences between 30% and 25% were not significant. The differences between 30% and 20% were significant at 5% level only.

ACKNOWLEDGEMENT

My grateful thanks are due to Professor L. S. S. Kumar, Economic Botanist and Principal, College of Agriculture, Poona, for guidance throughout the course of the investigations and the Indian Council of Agricultural Research, New Delhi, for financing the Scheme.

BIBLIOGRAPHY

Andrews, F. W. .. *Ann. App. Biology*, 1945, **32**, 193.
Bailey, L. F. .. *Amer. J. Bot.*, 1940, **27**, 122.
Brown, R. and Edwards, M. .. *Ann. Bot.*, 1944, N.S. **8**, 131.
———— .. *Nature*, 1945, **155**, 455.
Cochran, W. G. .. *Empire J. Expt. Agric.*, 1938, **6**, 157.
Davis, R. J. .. *J. Agric. Res.*, 1925, **31**, 801.
Dickson, J. G. .. *Ibid.*, 1923, **23**, 837.
Gilman, J. C. .. *Ann. Missouri Bot. Gdn.*, 1916, **3**, 25.
Johnson, J. and Hartmann, R. D. .. *J. Agric. Res.*, 1919, **17**, 41.
Jones, L. R. .. *Ibid.*, 1923, **26**, 459.
McKinney, H. H. .. *Ibid.*, 1925, **31**, 827.
Pearson, H. H. W. .. *Dept. Agric. Sci. Bull.*, Union of South Africa 1913, **40**.
Saunders, A. R. .. *Ibid.*, 1933, **128**.
Solomon, S. .. *Proc. Ind. Acad. Sci.*, 1952, **35**, 122.
Tate, P. .. *New Phytol*, 1925, **24**, 284.
Tims, E. C. .. *J. Agric. Res.*, 1926, **33**, 971.
Tisdale, W. H. .. *Ibid.*, 1917, **11**, 573.
Vallance, K. B. .. *Ann. Bot.*, 1950, N.S. **14**, 347.
———— .. *Ibid.*, 1951, N.S. **15**, 109.
Yates, F. .. *Empire J. Expt. Agric.*, 1933, **1**, 129.

EXPLANATION TO PLATES

Sorghum plants growing in Soil Temperature tanks :—

1. Left : Temperature .. 30° C.
   Moisture .. 20%
   Right : Temperature .. 35° C.
   Moisture .. 20%
2. Left : Temperature .. 35° C.
   Moisture .. 30%
   Right : Temperature .. 35° C.
   Moisture .. 40%

SORGHUM PLANTS GROWING IN SOIL TEMPERATURE TANKS

Temp. 30° C. Moist. 20% Temp. 35° C. Moist. 20%

1

V 36 1952 XUM

Temp. 35° C. Moist. 30% Temp. 35° C. Moist. 40%

2

AN ECTOPARASITE ON THE LARVAE OF *ANTHRENUS VORAX* WATERHOUSE

By P. K. Ayyappa and P. S. Cheema
(Technical Development Establishment Laboratories, Kanpur)

Received February 15, 1952
(Communicated by Dr. T. S. Subramanian)

1. Introduction

A parasite on *Anthrenus vorax* Waterhouse similar in behaviour to *Laelius voracis* Muesebeck (Muesebeck, 1939; Back, 1940) was spotted in Kanpur in May 1950 by one of the authors (P. K. A.) and has since been reared in these laboratories. There has been some divergence of opinion regarding the identity of the parasite. Dr. I. H. H. Yarrow of the British Museum considers the parasite to be possibly *Laelius elisae* Russo. In the absence of material for direct comparison Dr. Yarrow could not be certain of identity but he is of the opinion that " it is not, however, *L. voracis* Muesebeck bred in West America from *Anthrenus vorax*, and considering the lack of knowledge of Bethylids in general, may well turn out to be an undescribed species". Dr. K. V. Krombein of the U. S. National Museum, Washington, is also of the opinion that the parasites " represent an undescribed species of *Laelius* ". On the other hand, Prof. M. S. Mani, School of Entomology, St. John's College, Agra (India), considers the parasite to be *Laelius voracis* Muesebeck.

The host insect *Anthrenus vorax* is active throughout the year. A female lays on an average 36 eggs in her life-time. Eggs hatch in 8 days at 30° C. and the larvae which feed on woollen fabrics, hogs bristles, horns and other similar materials, complete their development in about 330 days. The pupal period at this temperature is about 9 days. The larvae constitute the host.

2. Life-History of the Parasite

(*a*) *The adult.*—The female parasite (Fig. 1) generally selects a medium-sized to full-grown larva of the host, clings on to the dorsum by the hair, curls the abdomen, paralyses the host by stinging it on the ventral side of the thoracic region and lays eggs. The paralysed larvae die later. Occasionally, a strong well-developed host larva may move out of the grip but is ultimately overcome by the parasite. Frequently, a number of larvae is paralysed and killed in succession without being oviposited on. Such larvae are kept for feeding purposes.

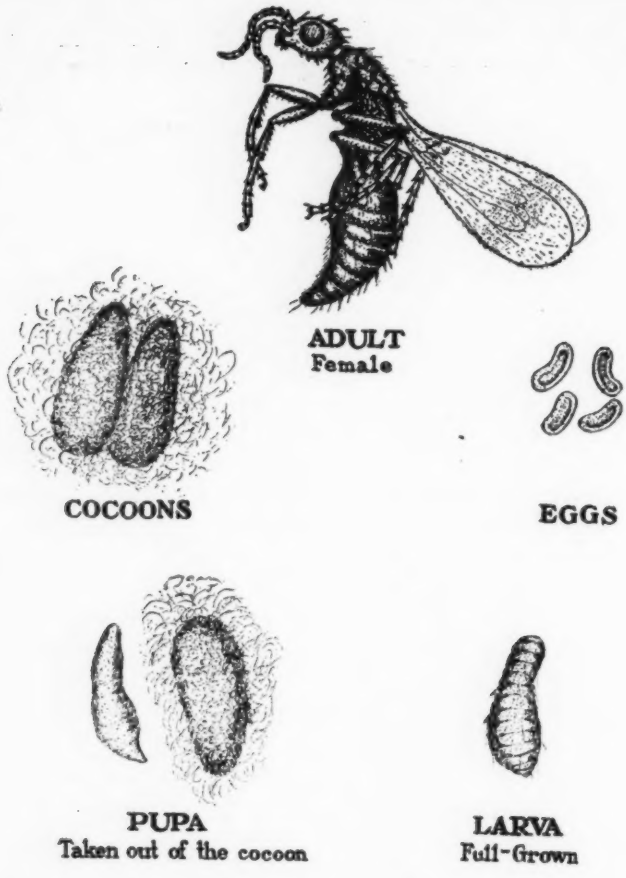


FIG. 1 (*Diagrammatic*)

Life-history of the parasite on the larva of *Anthrenus vorax* Waterhouse (Woolly bear)

(*b*) *Longevity of the adult*.—The males and females live on the average for about 5 and 35 days respectively (*vide* Table I).

(*c*) *Sex ratio*.—In order to determine the sex ratio, the progeny of seven females were examined. The results are recorded in Table II below.

The number of females is invariably more than that of males in the progeny of each female. In all, out of 365 adults produced by 7 females 65% were females and 35% were males and the sex ratio of female to male is, therefore, nearly 2: 1.

TABLE I

Longevity of Male and Female Parasites at 87 ± 2° F./84 ± 8% R.H.

Sl. No.	Life of male	Life of female
	No. of days lived	No. of days lived
1	3	24
2	2	26
3	3	28
4	10	20
5	5	20
6	2	32
7	3	47
8	6	37
9	4	59
10	6	27
11	7	41
12	8	37
13	4	38
14	5	58
Average	5	35

TABLE II

Sex Ratio of the Parasite

Serial No. of female *v*	Number of females produced	Number of males produced	Sex Ratio Female–Male (Approximate)
1	33	14	2:1
2	33	8	4:1
3	37	29	1:1
4	30	15	2:1
5	44	24	2:1
6	35	21	2:1
7	26	16	2:1
	238	127	2:1

(*d*) *Behaviour of the adult parasite towards the host larvae of different sizes.*—The sizes of freshly hatched and full-grown larvae of *A. vorax* are about 0·603 mm.×0·286 mm. and 4·714 mm.×2·014 mm. respectively.

Generally half-grown to full-grown larvae of the host are attacked by the parasite and eventually used for oviposition but in their absence even much younger larvae are attacked (Table III).

TABLE III

Behaviour of the Adult Parasite Towards Host Larvae of Different Sizes

Size of the host larvea		Observations
Length mm.	Thickness mm.	
0·764	0·320	Larva killed by biting
0·906	0·346	do.
0·960	0·500	do.
1·000	0·506	do.
1·333	0·560	do.
1·360	0·506	do.
1·466	0·573	do.
1·666	0·746	do.
1·733	0·707	Larva paralysed by stinging, leading to its death
1·933	0·773	Larva paralysed, killed and used for oviposition
2·066	0·866	do.
2·266	1·000	do.
4·500	2·000	do.

It is seen from the above table that very young larvae which measure less than 1·733 mm.×0·707 mm. are killed only by biting while larger larvae are paralysed by stinging and then used for depositing eggs.

(*e*) *Pre-oviposition, oviposition and post-oviposition periods of the parasite.*—Copulation takes place immediately after emergence of the adults from the cocoons. Although the female parasite starts stinging the host soon after emergence, it is some time before it starts laying eggs on the paralysed larvae. The females continue to live for some time after laying the last egg. The averages of fourteen observations in respect of pre-oviposition, oviposition and post-oviposition periods were 3, 31 and 4 days respectively at 87 ± 2° F./84 ± 8% R.H.

(*f*) *Number of larvae paralysed and used for oviposition by a single parasite and the number of eggs laid by a parasite.*—Before laying eggs, a female stings and paralyses some host larvae and removes the hair from certain parts

of the ventral surface of the thoracic region. The eggs are laid on the ventral side on the second or third thoracic segment with the narrow end of the egg attached to the body of the host. Observations were made at 87 ± 2° F./84 ± 8% R.H. on (i) the number of larvae paralysed and killed, (ii) the number of larvæ parasitised and (iii) the number of eggs laid by a single parasite. The results are shown in Table IV.

TABLE IV

Number of Larvae Paralysed and Used for Oviposition by a Single Parasite and the Number of Eggs Laid by a Single Parasite at 87 ± 2° F./84 ± 8% R.H.

Sl. No. of female parasite	Number of host larvae		Number of eggs laid
	Paralysed and killed	Parasitised	
1	96	23	35
2	119	24	54
3	139	23	55
4	117	21	46
5	120	26	50
6	142	34	61
7	187	61	90
8	175	34	62
9	253	67	100
10	134	46	75
11	189	50	81
12	174	32	57
13	162	44	57
14	224	58	73
Average	159	39	64

It is seen from the above table that a female is capable of paralysing and killing on an average 159 larvae in her life-time. Of these on an average 39 are used for laying about 64 eggs.

(*g*) *The egg.*—A female parasite lays 1–4 eggs on a single paralysed host (Fig. 2). Observations have shown that nearly 52% of host larvae had 1 egg, 30% had 2 eggs, 14% had 3 eggs and 4% had 4 eggs each. In rare cases, 5 eggs have also been found on one host larva. Larvae bearing varying number of eggs are shown in Fig. 2.

Freshly laid eggs are creamy white, with their posterior end broader than the anterior end and measure on average 0·471 mm.×0·185 mm. The

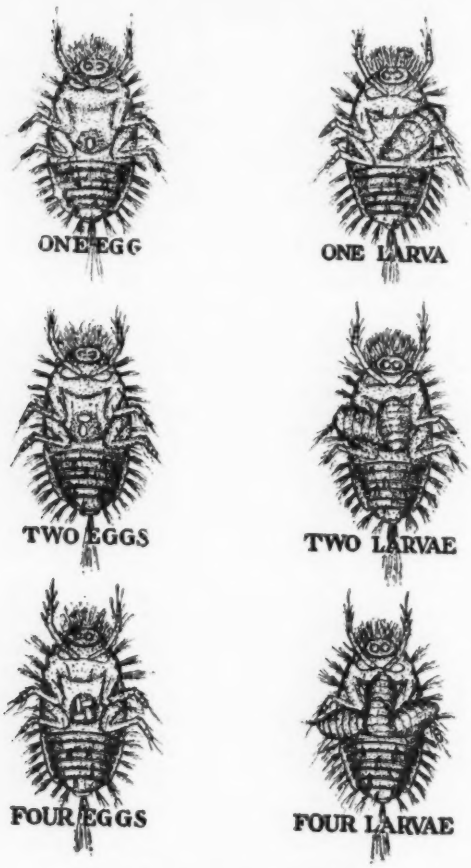


FIG. 2 (*Diagrammatic*)

Different number of eggs and larvae of the parasite on the woolly bear (Host) larvae

incubation period of 31 eggs laid on different larvae at 87 ± 2° F./84 ± 8% R.H. was found to be on an average, 2 days.

(*h*) *The larva.*—The larva, on hatching, measures on average 0·603 mm. × 0·286 mm. and starts feeding on the body fluid of the host by piercing the body at the third thoracic or the first or second abdominal segment, the exact position depending on where the egg was initially deposited. The larva continues feeding in the same position until it becomes full-grown when it measures 3·874 mm. × 1·326 mm. (Fig. 1). While feeding, the parasite larva keeps its head and almost whole of the thoracic region

inside the body of the host. The larval period occupies on an average 6 days at 87 ± 2° F./84 ± 8% R.H.

(*i*) *Development of more than one larva on one host.*—It has been already noted that the host larvae generally bear 1–4 eggs on their bodies. It was of interest to study whether all the larvae that hatch on a host develop successfully into cocoons. When 4 larvae are developing on one host, one or two fail to construct cocoons and thus die. When the number of larvae is less than 4 they are able to construct cocoons. Fig. 2 shows varying numbers of parasite larvae developing on hosts.

(*j*) *The pupa.*—When a parasite larva is fully grown, it constructs a white silken cocoon and pupates inside (Fig. 1). The pupa measures 3·450 mm. × 1·302 mm. The cocoons are seen attached to or lying close to the dead carcase of the host larva. The adult emerges by making a small hole at one end of the cocoon. On an average, the pupal period occupies about 21 days at 87 ± 2° F./84 ± 8% R.H.

Back's observations on *Laelius voracis* are as follows:—

> "The well-grown parasite larvae leave the host through openings made in the venter at about the union of the abdomen and thorax.... the white cocoons are usually spun by the larvae beneath or just to one side of the host, although they may be spun in cast larval skins of *A. vorax*, if these happen to be close to parasitised host; ....one to four parasites develop in a single well-grown *vorax* larva."

(*k*) *Parthenogenesis.*—The parasite shows parthenogenesis. Observations have shown that the unmated females always lay eggs and that the progeny are all males.

(l) *Alternate host.*—The parasite also attacks the larvae of *Attagenus* sp., under laboratory (artificial) conditions. In a mixed population of larvae of *A. vorax* and *Attagenus* sp. the parasite always prefers to attack larvae of *A. vorax* of sizes described in Table III. The relationship of the adult parasite towards the larvae of *Attagenus* sp. is similar to that towards the larvae of *A. vorax*. The development period of the parasite is about the same on both the hosts.

(*m*) *Hyperparasitism.*—So far no hyperparasite has been observed on this parasite.

4. Summary

A Bethylid parasite was spotted on the larvae of woolly bear, *A. vorax* in May, 1950. Its life-history has been studied in detail at 87 ± 2° F./84 ± 8% R.H. A female parasite generally selects a medium-sized to full-

grown larva and stings the latter on the ventral side of the thoracic region. The larva is thus paralysed and finally dies. Very young larvae which measure less than 1·733 mm.×0·707 mm. are killed by biting. The males live for about 5 days but the females live for 35 days. Sex ratio of female to male is nearly 2:1. Pre-oviposition, oviposition and post-oviposition periods have been observed to be 3, 31 and 4 days respectively. A female is capable of paralysing and killing on an average 159 larvae in her life-time. Of these only about 39 larvae are used for egg-laying. A female parasite lays 1–4 eggs (rarely 5) on a single paralysed host and in her life-time lays about 64 eggs. Eggs hatch within 2 days and the larvae pierce the body of the host and start feeding on the body fluid. Generally, three larvae are able to develop successfully into cocoons on one host. Larval period occupies about 6 days. The full-grown larva constructs a white silken cocoon and pupates inside. Pupal period occupies 21 days. Parthenogenesis occurs but the progeny are only males. Under laboratory conditions, the parasite attacks larvae of *Attagenus* sp. in the absence of larvae of *A. vorax*. So far no hyperparasite has been observed.

5. ACKNOWLEDGMENT

The authors' thanks are due to Dr. T. S. Subramanian, Superintendent of Development, Technical Development Establishment Laboratories, Kanpur, for his interest in the work. This paper is published with the permission of the Director of Technical Development, and Master General of the Ordnance, Army Headquarters, New Delhi.

6. REFERENCES

1. Muesebeck, C. F. W. .. "The North American species of the genus *Laelius* Ashmead (Hymenoptera : Bethylidæ)," *Biol. Soc. Wash. Proc.*, 1939, **52**, 171–76.

2. Back, E. A. .. "A new parasite of *Anthrenus vorax* W.," *Proc. Ent. Soc. Wash.*, 1940, **42** (5), 110–13.

EXPLANATION OF PLATE

FIG. 3. Eggs of the parasite, × 25.

FIG. 4. A. Host larva bearing one parasite larva, × 10.
B. Host larva bearing two parasite larva, × 10.

FIG. 5. Host larva bearing four parasite larva, × 10.

FIG. 6. A. Pupa of the parasite taken out of the cocoon, × 15.
B. Cocoon of the parasite, × 15.

937-52 Printed at the Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore.

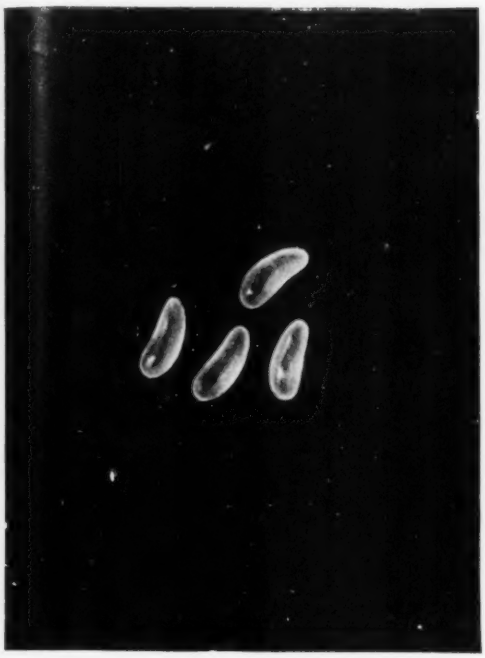


FIG. 3

FIG. 4

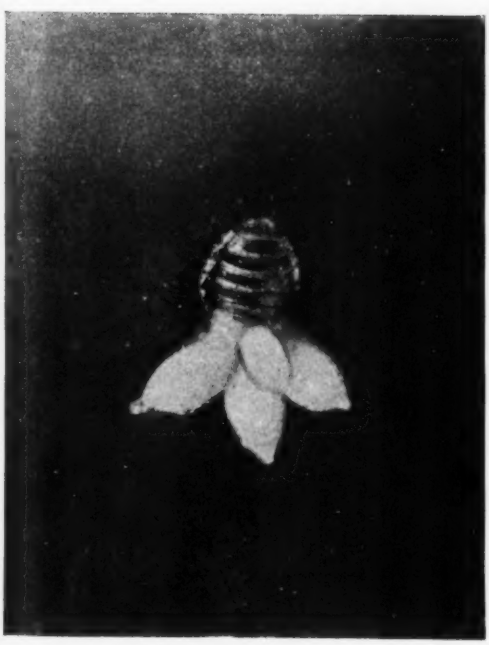


FIG. 5

FIG. 6

V 36 1952 XUM

ERRATA

Vol. XXXVI B, No. 4, October 1952

Page 151, legend for Fig. 2

line 3 *for* 25° C. ■. *read* 28° C. ■.

line 4 *for* 25° C. × *read* 25° C. ⊗.

Page 155, line 27 *for* "length-weight equation", *read* "length-oxygen consumption equation".

Page 155, line 35 *for* X_6 *read* X_1

Page 156, line 16 *for* *x* *read* X.

V 36 1952 XUM

FUNGI IMPERFECTI FROM MADRAS—III

Beltraniella gen. nov.

By C. V. Subramanian

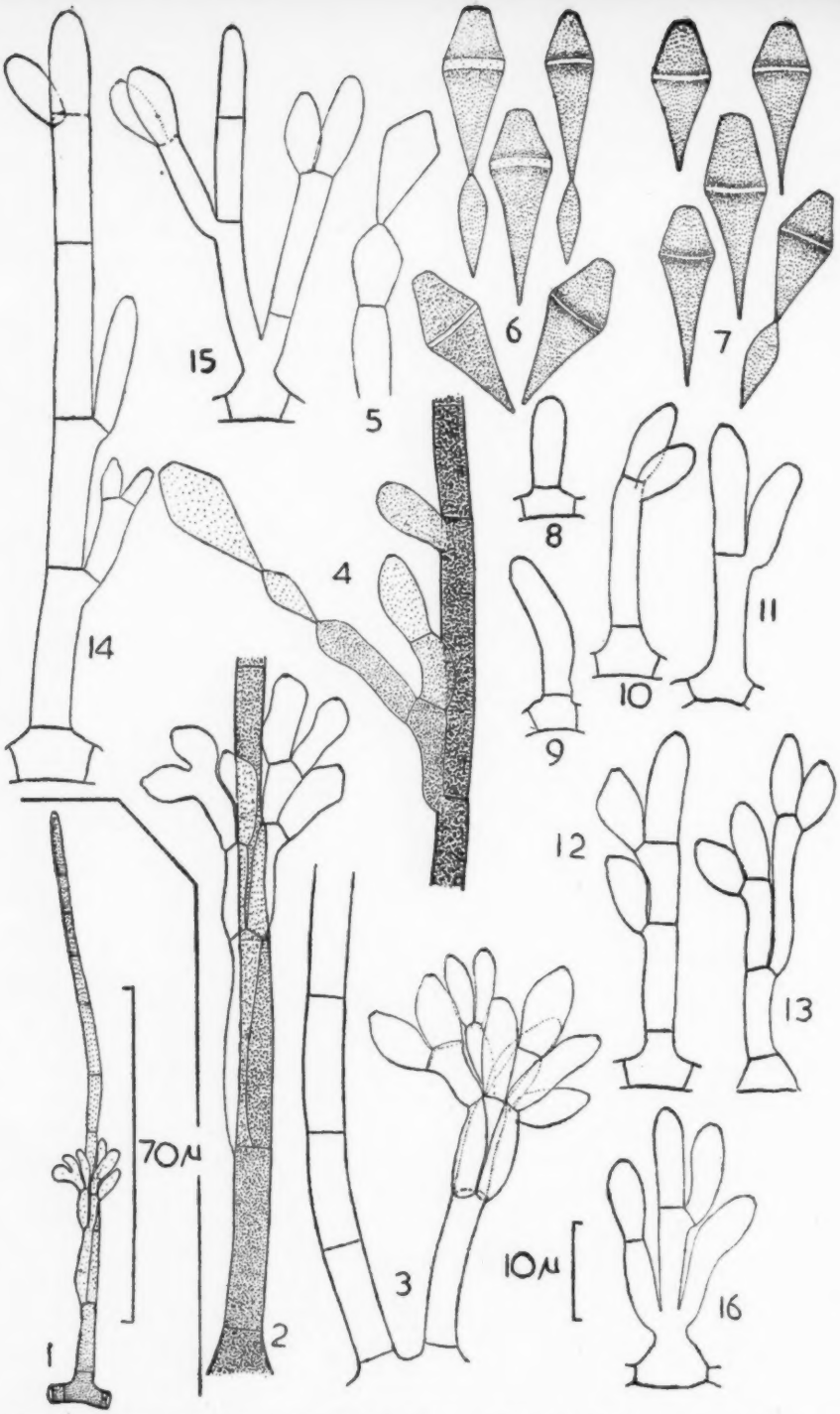
(*University Botany Laboratory, Madras*)

Received November 5, 1952

(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

Amongst the fungi recently collected at Madras is an interesting Hyphomycete belonging to the Dematiaceæ. Two collections of the fungus were made, both on dead and decaying leaves of *Odina wodier* Roxb. and the description given below is based on these two collections.

The fungus usually occurs on leaves in moist leaf litter. It forms effuse colonies on both sides of leaves. The colonies are bluish green when freshly collected, but turn brownish when dry. The conidiophores are solitary and arise as lateral branches from cells of repent hyphæ. A mature conidiophore (Fig. 1) consists of a seta-like, thick-walled, rigid main axis which is erect, straight, dark-coloured, paler in colour towards the base and darker coloured towards the apex, up to 10-septate, tapering to a fine pointed or sometimes rounded tip, 120–230 μ long, 3·5–7 μ broad at the base. One or more cells of this main axis, but not the apical one, bear lateral branches just below the cross walls (Figs. 1, 4, 14). These lateral branches may give rise to further secondary, tertiary or quaternary branches, the ultimate branches being the spore-bearing cells which bear the conidia (Figs. 2, 4, 5). The branches are separated by septa, but are themselves rarely septate. The primary and, to a less extent, the secondary lateral branches tend to be erect and parallel to the main axis. The secondary and the ultimate branches show gradations in colour, the latter being much paler than the former. The ultimate branches (spore-bearing cells) are subhyaline, thin-walled and smooth in outline and each of them has a single scar indicating the insertion of the conidium which has fallen away, and measure 8–15 × 3–5 μ. The conidia are deciduous and detached conidia have been seen in connection with smaller fusiform cells (Figs. 4, 6, 7), which I consider to be " separating cells ", somewhat similar to what have been described for various species of *Beltrania* (Hughes, 1951 *a*; Subramanian, 1952). In one case a young conidium was seen attached directly to the conidiophore (Fig. 5); it is, therefore, likely that some conidia may not have " separating cells " or that the " separating cell " may be formed after the conidium develops. The separating cell is deciduous, fusiform, smooth-walled, hyaline to subhyaline.



FIGS. 1-16. *Beltraniella odinæ*. Figs. 1, 2, 4 and 7 from Herb. M.U.B.L. No. 410; other figs. from Herb. M.U.B.L. No. 377. For explanation, see text.

somewhat sharply tapering to a pointed apex (on which the conidium is borne), gradually tapering to a pointed base (where it is attached to the spore-bearing cell), 11–18×3–5 μ (Fig. 4). A single separating cell is borne at the tip of each spore-bearing cell. The conidia (Figs. 6, 7) are acrogenous, produced on separating cells, top-shaped, smooth-walled, subhyaline to faintly coloured, one-celled with a pale band where they are broadest, attached by their pointed ends, deciduous. 23×7·6 (18–26×6–9) μ.

The development of the conidiophore appears to be as follows. The conidiophore arises as a lateral outgrowth from a cell of the repent hypha, and is hyaline when young. Each outgrowth elongates, and subsequently a cross wall is formed near its base (Fig. 8) separating it from the parent cell of the repent hypha. A cross wall is later formed about the middle of the cell (Fig. 10). Sometimes the formation of the first cross wall is delayed (Fig. 11). A lateral branch may then arise from the lower cell just below the cross wall (Figs. 10, 11, 12) and this may give rise to the secondary branches, etc. (Fig. 13). The main branch elongates further and also becomes many-septate, and more lateral branches arise from the cells of the main branch immediately below the cross walls (Fig. 14), and the lateral branches in turn produce secondary, tertiary branches, etc. The main branch elongates further and develops into the thick-walled seta-like main axis (Fig. 1). A section of the colonised leaf shows a large number of young conidiophores with fewer mature seta-like conidiophores interspersed between them.

The important characteristics of the fungus described above are: (i) the seta-like, thick-walled, dark-coloured, septate conidiophore bearing lateral branches; (ii) the " separating cells " borne singly at the apex of the spore-bearing cells; (iii) the top-shaped, subhyaline to faintly coloured, one-celled conidia (with a pale band where they are broadest) produced acrogeneously and attached singly by their pointed ends to the tips of the " separating cells ".

The presence of seta-like structures, " separating cells " and one-celled conidia with pale bands indicates resemblance to some species of the genus *Beltrania* Penzig (see Hughes, 1951 *a*). However, this resemblance is superficial. For, the setæ in *Beltrania* are sterile, and the conidiophores are usually unbranched and bear conidia acropleurogenously; in my fungus the conidiophores are seta-like and there are no sterile setæ. Further, in my fungus the conidiophores bear lateral branches, the conidia being produced acrogenously at the tips of the ultimate branches. My fungus is, therefore, distinct from *Beltrania*.

Other genera which were considered in the search for a suitable name for the fungus were: *Pseudobeltrania* Hennings; *Chætopsis* Greville; *Chætopsella* Hoehnel; *Mesobotrys* Saccardo; *Menispora* Pers.; *Menisporopsis* Hughes; *Monilochætes* Harter; and *Verticicladium* Preuss.

The genus *Pseudobeltrania* was established for *P. cedrelæ* by Hennings (1902). I have not seen a specimen, but the description (Hennings, 1902, p. 310) indicates that the conidiophores are inflated and the conidia are two-celled. My fungus is therefore not congeneric with *Pseudobeltrania*.

Of the other genera, *Chætopsis*, *Chætopsella*, *Mesobotrys* and *Verticicladium* have recently been discussed in some detail by Hughes (1951*b*, 1951*c*) after a careful study of specimens and the fact that these genera are therefore now better known minimises considerably the uncertainties usually accompanying comparisons based on descriptions alone. The genus *Chætopsis* Greville resembles my fungus in having conidiophores with a rigid, erect main axis and lateral branches; but it differs from my fungus in having two-celled conidia which are oblong-cylindric to cylindric and are produced in groups on the " phialides ". In my fungus a spore-bearing cell produces a single conidium acrogenously, and repeated production of conidia from the same spore-bearing cell as in the case of *Chætopsis* has not been seen. Further, unlike *Chætopsis*, the apex of the mature conidiophore is always sterile. *Chætopsella* Hoehnel, according to Hughes (1951 *b*), is probably a later synonym of *Chætopsis* and hence needs no further consideration in the present context. In *Mesobotrys* Sacc. the conidia, which are one-celled, are stated to be globose to ovoid and hence this genus again cannot accommodate my fungus. Moreover, *Mesobotrys* Sacc. is a later synonym of *Gonytrichum* Nees ex Wallroth (Hughes, 1951 *b*) and the morphology of this fungus, as described in detail by Hughes (1951 *b*), is so different from that of my fungus as to merit no further consideration here. The genus *Verticicladium* Preuss as typified by *V. trifidum* Preuss resembles my fungus in having a main stipe bearing lateral branches which ultimately end in a spore-bearing cell, but the apical cell of the stipe is fertile in *V. trifidum* and the conidia are oval and more than one conidium is produced from each spore-bearing cell. My fungus cannot therefore be placed in the genus *Verticicladium*.

The genus *Menispora* Pers. (Saccardo, 1886) is characterised by falcate conidia, and *Menisporopsis* (Hughes, 1952) by falcate conidia with appendages. Thus, either of these two genera cannot accommodate my fungus.

The genus *Monilochætes* Harter was taken up for comparison since it has been cited as a synonym of *Chætopsis* Greville by Clements and Shear

(1931, p. 393). The type species, *Monilochætes infuscans* (Ell. and Hals.) Harter produces, however, amerospores (Harter, 1916, p. 791) and is not congeneric with *Chætopsis* which has didymospores (Hughes, 1951 *b*). Further, in *Monilochætes infuscans* the conidia are produced acrogenously at the tips of unbranched conidiophores. My fungus, therefore, cannot be placed in this genus either.

In the light of the above facts it is considered necessary to place my fungus in a new genus. The name *Beltraniella* is chosen, from its superficial, but not true, resemblance to *Beltrania*.

Beltraniella Subramanian gen. nov.

Pertinent ad Fungos Imperfectos, Hyphomycetes, Dematiaceæ, Amerosporas. Hyphæ subhyalinæ vel fusce coloratæ, septatæ. Conidiophori erecti, prædíti axi principali instar setæ, crasse vallatæ, fusce coloratæ, septatæ, ramis lateralibus ornatæ. Rami laterales iterum ramosi, penultimæ ramificationes producentes conidia. Conidia turbini similia, subhyalina vel pallide colorata, uni-cellulata, acrogena, infixa cellulis separantibus per acutum apicem.

Species typica sequens.

14. **Beltraniella odinæ** Subramanian sp. nov.

Coloniæ effusæ, ceruleo-virides vel griseæ in statu juniori, brunneæ in statu seniori. Conidiophori axi principali instar setæ prædíti ramos laterales ferentis. Axis erectus, rectus, latus ad basim, desinens in acutum vel obtusum apicem, subhyalinus vel brunneus colore, fuscior ad apicem, usque decies septatus, crasse parietatus, 120–230 μ longus, 3·5–7 μ latus ad basim. Rami pallidiores colore quam axis, emergentes ex una vel pluribus cellulis axis (una excepta cellula apicali) immediate sub septa, iterum producentes ramificationes secondarias, tertiarias, vel quaternarias, 3–7 μ lati, longitudine variante. Conidia acrogena, singula ex cellula separante fusiforme producta, turbini similia, subhyalina vel pallide colorata, uni-cellulata, infixa per apices acutos, decidua, $23 \times 7 \cdot 6$ ($18–26 \times 6–9$) μ.

Habitat in foliis emortuis *Odinæ wodieri* Roxb.; typus lectus in campo Botany Laboratory, Madras a K. Ramakrishnan and C. V. Subramanian die 25 mensis augusti 1951 et positus in herb. M.U.B.L. No. 377; lectus etiam a K. Ramakrishnan die 29 augusti 1952, Herb. M.U.B.L. No. 410.

Beltraniella Subramanian gen. nov.

Fungus imperfectus, hyphomycete, Dematiaceæ, amerosporæ. Hyphæ subhyaline to dark-coloured, septate. Conidiophore with a seta-like erect,

thick-walled, dark-coloured, septate main axis bearing lateral branches. Lateral branches further branched, the ultimate branches bearing the conidia. Conidia top-shaped, sub-hyaline to faintly coloured, one-celled, acrogenous, attached by their pointed ends to " separating cells ".

Type species:

Beltraniella odinæ Subramanian sp. nov.

Colonies effuse, bluish green to gray when fresh, brownish when dry. Conidiophore with a seta-like main axis bearing lateral branches. Axis erect, straight, broad at the base, tapering to a rounded or pointed apex, sub-hyaline to brownish in colour, darker coloured towards the apex, up to ten-septate, thick-walled, 120–230 μ long, 3·5–7 μ broad at the base. Branches paler in colour than the axis, arising from one or more cells of the axis (except the apical cell) just below the septa, giving rise to secondary, tertiary or quaternary branches, 3–7 μ broad, of variable length. Conidia acrogenous, produced singly on a fusiform " separating cell ", top-shaped, sub-hyaline to faintly coloured, one-celled, attached by their pointed ends, deciduous, $23 \times 7 \cdot 6$ $(18–26 \times 6 \cdot 9)$ μ.

Habit: on decaying and dead leaves of *Odina wodier* Roxb., University Botany Laboratory campus, Madras; coll. K. Ramakrishnan and C. V. Subramanian, 25–8–1951 (Type) (Herb. M.U.B.L. No. 377); coll. K. Ramakrishnan, 29–8–1951 (Herb. M.U.B.L. No. 410).

I am grateful to Professor T. S. Sadasivan for critically reading the manuscript and to Professor H. Santapau for the Latin diagnoses.

REFERENCES

Clements, F. E. and Shear, C. L. .. *The Genera of Fungi*, 1931, 496 pp. The H. W. Wilson Co., New York.

Harter, L. L. .. "Sweet potato scurf," *J. agric. Res.*, 1916, **5**, 787–91.

Hennings, P. .. "Fungi S. Paulensis. II. a cl. Puttemans collecti," *Hedwigia*, 1902, **41**, 295–311.

Hughes, S. J. .. "Studies on micro-fungi, XIII," *Mycol. Pap. C.M.I.*, 1951 *a*, **47**, 15 pp.

——— .. "*Stachylidium*, *Gonytrichum*, *Mesobotrys*, *Chætopsis* and *Chætopsella*," *Trans. Brit. mycol. Soc.*, 1951 *b*, **34**, 551–76.

——— .. "Studies on micro-fungi, IX, *Mycol. Pap. C.M.I.*, 1951 *c*, **43**, 25 pp.

——— .. "Fungi from Gold Coast I," *ibid.*, 1952, **48**, 91 pp.

Saccardo, P. A. .. *Sylloge Fungorum*, 1886, **4**.

Subramanian, C. V. .. "Fungi Imperfecti from Madras — I," *Proc. Indian Acad. Sci.*, 1952, **36 B**, 43–53.

SOIL CONDITIONS AND ROOT DISEASES

VI. Germination of Conidia of *Fusarium vasinfectum* in Micro-Element Amended Soils

By Miss C. B. Sulochana

(*University Botany Laboratory, Madras*)

Received November 17, 1952

(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

Introduction

Germination of fungal spores is an important aspect of all plant pathological work, since spore germination is a precursor of infection. Anti-sporulating effects of certain micro-elements on *Fusarium vasinfectum* observed in the studies of response of cotton-wilt fungus to micro-elements in pure culture (Sadasivan, 1951, Table V) and the effects of micro-element amendments on the saprophytic potentialities of Fusaria in soil (Sulochana, 1952) prompted a study of germination of micro- and macro-conidia of *Fusarium vasinfectum* in soil *in situ* in the presence of micro-elements, since a knowledge of the conditions influencing germination of fungal spores is of considerable practical importance.

Materials and Methods

Four microscopic slides smeared uniformly on one side with *F. vasinfectum* spore suspension in 2% water-agar and air-dried (modified Cholodny's slide technique—Cholodny, 1930) were buried in each glass tumbler containing 200 gm. of Udamalpet cotton soil. Aluminium, boron, cobalt, lithium, manganese, molybdenum, nickel and zinc at 50, 100, 200, and 400 p.p.m. levels were used to amend the soil samples. Moisture level was adjusted to 50%. Slides were carefully removed after 48 and 72 hours' incubation respectively and stained according to standard methods and then air-dried. Counts of germinated and ungerminated spores in ten randomised microscopic fields were made for each slide. Results were tabulated on percentage basis and analysed statistically.

Experimental Results

Results are presented in Tables I, II and III. Table I shows the percentage germination of spores at the various concentrations with four replications for each. Analysis of Variance of Table II showed that the

TABLE I

Percentage Germination of Micro- and Macro-Conidia of Fusarium vasinfectum *in Soils—Udamalpet 'Wilt-Sick' Soil Amended with Micro-Elements (Cholodny Slide Technique)*

Micro-elements		Incubation period in hours	Concentration of Micro-Elements															
			50 p.p.m.				100 p.p.m.				200 p.p.m.				400 p.p.m.			
			Replicates				Replicates				Replicates				Replicates			
			I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Aluminium	1	48	35	33	40	26	33	30	30	28	10	15	5	16	7	9	5	7
	2	72	40	38	20	24	31	34	24	26	14	10	12	6	10	5	5	11
Boron	1	48	45	51	40	46	40	31	28	42	11	6	18	14	10	7	13	10
	2	72	52	50	48	40	45	20	25	34	12	15	10	13	9	15	11	8
Cobalt	1	48	58	60	64	47	45	49	31	42	44	40	34	38	20	18	16	18
	2	72	60	65	54	40	52	36	39	48	41	41	44	36	16	19	21	20
Lithium	1	48	30	31	22	27	20	22	10	14	10	12	14	16	4	3	11	7
	2	72	40	45	44	36	17	20	19	22	9	15	20	10	10	10	5	8
Manganese	1	48	84	66	71	74	60	65	50	52	35	30	18	22	11	10	12	11
	2	72	79	40	69	80	64	61	59	53	37	40	52	28	15	16	18	14
Molybdenum	1	48	22	24	30	31	22	18	20	20	5	4	4	6	3	4	6	2
	2	72	37	42	40	45	34	36	22	31	10	9	7	6	6	6	8	7
Nickel	1	48	25	20	22	21	23	26	26	25	20	21	21	19	11	5	7	7
	2	72	24	25	26	22	30	21	32	24	23	24	20	24	9	12	14	11
Zinc	1	48	18	20	25	31	20	22	22	21	8	4	3	7	2	5	4	8
	2	72	40	42	45	37	25	26	20	24	10	12	14	10	6	8	8	9
Control	1	48	68	71	56	70												
	2	72	75	80	64	76												

TABLE II

TABLE II

Analysis of Variance

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Log_{10} V	Value of Z		
					Calculated	At 5% point	At 1% point
Micro-elements	7	18936·96	2705·28	3·4322154	3·15181	·3885 (6,120)*	·541 9 (6,120)
Concentrations	4	140197·24	35049·31	4·5446756	4·54772	·4475 (4,120)	·6234 (4,120)
Incubation period	1	1272·98	1272·98	3·1048284	2·77489	·6830 (1,120)	·9622 (1,120)
Interactions: Elements × Concentrations ..	28	11442·26	408·65	2·6113515	2·20675	·2376 (24,120)	·3339 (24,120)
Concentrations × Incubation periods	4	313·16	78·29	1·8937063	1·38053	·4475 (4,120)	·6234 (4,120)
Elements × Incubation periods	7	1000·31	142·90	2·1550322	1·68139	·3885 (6,120)	·5419 (6,120)
Elements × Concentrations × Incubation periods	28	15241·44	544·34	2·7358702	2·35011	·2376 (24,120)	·3339 (24,120)
Error	240	1187·75	4·95	0·6946052			
TOTAL ..	319	189592·10					

* Degrees of freedom for treatments and error (nearest values from 'Z' table).

TABLE III

Germination of F. vasinfectum *Spores in Micro-Element Amended 'Wilt-Sick' Soil*

Analysis of Variance

Factor	Degrees of freedom	Sum of squares	Variance
Whole unit treatment ..	31	2010·40	
Micro-elements ..	7	18936·96	2705·28
Error ..	24	1164·44	48·59

Critical difference at 5% level $= \sqrt{48 \cdot 59 \times 2 \times 40} \times 2 \cdot 064 = 128 \cdot 67$

Total Percentage Counts

Manganese (A) ..	1956	Aluminium (E) ..	1199
Cobalt (B) ..	1816	Lithium (F) ..	1143
Boron (C) ..	1379	Molybdenum (G) ..	1124
Nickel (D) ..	1200	Zinc (H) ..	1116

A B C $\overline{\text{D E F G H}}$

effects of micro-elements, concentrations, incubation periods, the double interactions and the triple interactions were significant at both 5% and 1% points inasmuch as the percentage germination of *Fusarium vasinfectum* spores in all the micro-element amended soil samples excepting the manganese-treated soil at 50 p.p.m. concentrations was much lower than that of the control.

The order of the micro-elements in relation to their inhibitory effect on spore germination in the descending order would be zinc, molybdenum, aluminium, nickel, boron, cobalt and manganese (Table III). The inhibitory effect on spore germination was found to be directly proportional to the increase in concentrations. Higher concentrations of the various micro-elements recorded very low percentage germination of *F. vasinfectum* spores.

Manganese at 50 p.p.m. concentration was actually found to stimulate conidial germination. Toxic effect was observed only at 200 p.p.m. and higher concentrations of manganese.

In several cases longer incubation period resulted in higher percentage spore germination, presumably due to the depletion of the concentration of heavy metal amendment by the growth and activity of the mixed general

microflora of the soil. However, this point needs more critical observations before any firm conclusion can be arrived at.

Discussion

This study signifies further the importance of amending wilt-infected soil with micro-elements, as the changes brought about in the soil conditions, retarded considerably the germination of *Fusarium vasinfectum* spores introduced into the soil and possibly will control the development, sporulation and propagation of the pathogen in soil markedly. The results presented herein also indicate scope for the investigation of behaviour and survival of conidia of soil-borne pathogenic fungi in the presence of trace-elements.

Summary

A study of the germination of micro- and macro-conidia of *Fusarium vasinfectum* introduced into the wilt infected soils amended with micro-elements was made employing modified Cholodny's slide technique.

Statistical analysis of the results showed the micro-elements, concentrations, incubation periods and the interacting factors to be highly significant from the point of view of retardation of conidial germination.

Percentage germination of the conidia in all the amended soil samples, excepting manganese 50 p.p.m., was much lower than that of the unamended control soil. Zinc, molybdenum, lithium, aluminium, nickel, boron, cobalt and manganese were correspondingly inhibitive in the order mentioned.

Acknowledgements

The author expresses her indebtedness to Professor T. S. Sadasivan and thanks the University of Madras for award of a stipend during the tenure of which this investigation was conducted.

References

*Cholodny, N. .. "Ueber eine neue methods zur Untersuchung der Bodenmikroflora," *Arch. Mikrobiol.*, 1930, **1**, 620.

Sadasivan, T. S. .. "Role of trace elements in control of root-infecting fungi," *Proc. Indian Acad. Sci.*, 1951, **33 B**, 135–49.

Sulochana, C. B. .. "Soil conditions and Root Diseases, III with special reference to colonisation and survival of soil Fusaria in soils treated with micro-elements," *ibid.*, 1952, **35 B**, 209–13.

* Original not seen.

SOIL CONDITIONS AND ROOT DISEASES

VII. Response of Cotton Plants to Micro-Element Amendments and Its Relation to Disease Development

By Miss C. B. Sulochana

(*University Botany Laboratory, Madras*)

(Received November 17, 1952)

(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

Introduction

Having made independent studies on response of the cotton wilt fungus *Fusarium vasinfectum* to a substratum containing micro-elements in pure culture and on saprophytic potentialities of this pathogen in micro-element amended soils in the presence of diverse microflora it was found necessary to study the effects of micro-elements on the cotton plant development and disease incidence. The results of such an experimental lay-out are presented here.

Materials and Methods

Udamalpet (Coimbatore District, Madras State) cotton field soil samples were amended with aluminium, boron, cobalt, lithium, manganese, molybdenum, nickel and zinc at 50, 100, 200 and 400 p.p.m. concentrations. Treatments were run in triplicates. Healthy cotton seeds of a susceptible variety of cotton K.I. pure (97–100% germination) were used throughout. 25 delinted surface-sterilised seeds were sown for each treatment. Germination counts were taken up to 15 days following the sowing date. Green and dry weights of the shoots were taken six weeks after sowing.

For pathogenicity tests, pots were incubated for four weeks at 50% moisture level on additions of micro-elements to the soil samples, after which 10% by weight of oat soil inoculum (Padwick, 1940) was added to each pot and mixed well with the upper one inch layer of the soil. After 24 hours' incubation selected good cotton seeds were sown. Percentage pre-emergence and post-emergence wilt were calculated based on the number of plated (ungerminated seeds) and wilted seedlings giving *Fusarium vasinfectum*.

Experimental Results

Percentage germination was 100 in the control and all the treated soils except in the case of boron treatment series.

TABLE I

Germination Percentage of Cotton Seeds (K.1.) in "Wilt-Sick" Soils Amended with Micro-Elements

Micro-elements	Concentration in p.p.m.	Percentage germination of seeds
Aluminium ..	50	100
	100	100
	200	100
	400	100
Boron ..	50	92
	100	60
	200	4
	400	..
Cobalt ..	50	100
	100	100
	200	100
	400	100
Lithium ..	50	100
	100	100
	200	100
	400	100
Manganese ..	50	100
	100	100
	200	100
	400	100
Molybdenum ..	50	100
	100	100
	200	100
	400	100
Nickel ..	50	100
	100	100
	200	100
	400	100
Zinc ..	50	100
	100	100
	200	100
	400	100
Control ..		100

TABLE II

The Effects of Micro-Element Amendments to "Wilt-Sick" Soil on Cotton Plant Growth

(Six weeks growth after sowing)

		Height in inches	Green weight in gm.	Dry weight in gm.	Condition of plants
Aluminium	50 p.p.m. ..	7·0	5·38	0·85	Normal healthy
	100 ,, ..	6·2	5·02	0·80	do.
	200 ,, ..	5·5	3·55	0·54	do.
	400 ,, ..	5·5	3·28	0·505	do.
Boron	50 ,, ..	6·0	4·29	0·71	Slight boron toxicity (Marginally scorched leaves)
	100 ,, ..	4·5	2·93	0·38	Severe boron toxicity
	200 ,, ..	..	..	..	..
	400 ,, ..	..	..	..	..
Cobalt	50 ,, ..	7·5	5·32	0·83	Normal
	100 ,, ..	7·5	5·31	0·81	do.
	200 ,, ..	7·1	5·08	0·72	do.
	400 ,, ..	6·6	5·00	0·718	do.
Lithium	50 ,, ..	4·3	4·05	0·66	Slight toxicity symptoms (Marginally scorched leaves)
	100 ,, ..	4·3	4·02	0·58	Severe toxicity symptoms
	200 ,, ..	4·1	3·4	0·41	do.
	400 ,, ..	4·1	3·0	0·36	Very severe toxicity symptoms
Manganese	50 ,, ..	8·8	5·82	0·91	Normal healthy
	100 ,, ..	8·65	5·46	0·89	do.
	200 ,, ..	8·6	4·97	0·799	do.
	400 ,, ..	8·4	4·925	0·73	do.
Molybdenum	50 ,, ..	5·0	3·85	0·53	Stunted seedlings with small crowded leaves
	100 ,, ..	4·2	3·51	0·51	do.
	200 ,, ..	4·10	2·02	0·408	do.
	400 ,, ..	3·2	1·75	0·32	Stunted with very few leaves
Nickel	50 ,, ..	8·4	4·48	0·71	Normal
	100 ,, ..	8·0	4·46	0·682	do.
	200 ,, ..	7·6	4·21	0·64	do.
	400 ,, ..	7·2	4·03	0·60	do.
Zinc	50 ,, ..	10·2	6·88	1·35	Normal very healthy
	100 ,, ..	9·6	6·49	1·28	do.
	200 ,, ..	8·8	5·95	1·09	do.
	400 ,, ..	8·6	5·91	1·07	do.
Control	.. ..	6·9	4·63	0·695	Normal

Although 92% germination was recorded at 50 p.p.m. of boron, the germination was much delayed. At 100 p.p.m. of boron, more than 50% of germinated seeds failed to show complete growth. Only the hypocotyls were seen above ground. No seed showed full germination at 200 p.p.m., and at 400 p.p.m., there was no germination.

Distinct improvement was observed in seedling growth in zinc, manganese, and nickel treatments and also at lower levels of cobalt and at 50 p.p.m. of aluminium.

Boron and lithium produced toxic symptoms. Severe marginal scorching and yellowing of leaves were observed even at 50 p.p.m. concentration. In molybdenum treatments, the seedlings were much stunted in growth, with small pale yellow leaves crowded at the apex.

An appreciable increase in green and dry matter was recorded in zinc and manganese treatments.

Table III shows the values recorded in a subsequent experiment made on the effects on germination and vigour of seedlings on soaking delinted surface sterilised seeds in various strengths of micro-elements for 8 and 16 hours respectively, prior to sowing—particularly to study the behaviour of boron treatments.

100% germination was observed in zinc, manganese, molybdenum, aluminium and boron treatments. Lithium and cobalt showed fairly high percentage germination. Nickel treatments showed maximum inhibition of germination.

In this series also zinc showed the highest green weights, closely followed by manganese.

It is of interest to note that the seeds soaked in boron solutions of various strengths did not develop any toxic symptoms, whereas when added to soil even at low concentrations severe toxicity and inhibition of germination resulted. Apparently the toxic symptoms are not the result of direct boron poisoning. It may be due to changes brought about in the soil conditions, particularly those of a chemical nature, since it has been shown by Brenchley and Warrington (1927) that in plants the action of boron is intimately connected with the function of calcium.

Nickel was toxic when the seeds were soaked in nickel sulphate solution, but not so when the soil was amended with nickel (Tables III and IV).

No pre-emergence wilt was observed in aluminium, cobalt, lithium, molybdenum, nickel and zinc treatments. At 50 p.p.m. of boron and all the levels of manganese, pre-emergence wilt occurred to a considerable

TABLE III

The Effects of Presoaking Cotton Seeds in Micro-Element Solutions on Germination and on Seedling Vigour and Growth

(Six weeks' growth after sowing)

Treatments		PERIOD OF SOAKING					
		8 Hours			16 Hours		
		Percentage germination of cotton seeds	Green wt. in gm.	Condition of plant	Percentage germination of cotton seeds	Green wt. in gm.	Condition of plant
Aluminium	50 p.p.m. ..	100	5·38	Normal	100	5·44	Normal
	100 ,, ..	100	5·31	do.	100	5·38	do.
	200 ,, ..	100	5·26	do.	100	5·35	do.
	400 ,, ..	100	4·99	do.	100	5·01	do.
Boron	50 ,, ..	100	5·291	do.	100	5·30	do.
	100 ,, ..	100	5·25	do.	100	5·08	do.
	200 ,, ..	100	5·18	do.	100	5·00	do.
	400 ,, ..	100	5·09	do.	100	4·79	do.
Cobalt	50 ,, ..	100	3·74	Slightly stunted	100	3·63	Slightly stunted
	100 ,, ..	100	3·80	do.	100	3·39	do.
	200 ,, ..	100	3·62	do.	100	3·27	do.
	400 ,, ..	100	3·35	do.	100	2·09	do.
Lithium	50 ,, ..	100	5·12	Normal	100	4·95	Normal
	100 ,, ..	100	5·03	do.	100	4·93	do.
	200 ,, ..	100	4·6	do.	100	4·69	do.
	400 ,, ..	100	4·54	do.	100	4·58	do.
Manganese	50 ,, ..	100	7·76	do.	100	7·36	do.

Manganese	50 ,, ..	100	7·76	do.	100	7·36	do.
	100 ,, ..	100	7·38	do.	100	7·30	do.
	200 ,, ..	100	7·33	do.	100	7·05	do.
	400 ,, ..	100	7·05	do.	100	7·02	do.
Molybdenum	50 ,, ..	100	5·88	Normal	100	5·32	Normal
	100 ,, ..	100	5·84	do.	100	5·31	do.
	200 ,, ..	100	5·61	do.	100	5·05	do.
	400 ,, ..	100	5·38	do.	100	5·01	do.
Nickel	50 ,, ..	20	3·75	Stunted seedlings	10	1·38	Sickly stunted seedlings
	100 ,, ..	8	3·03	do.	..*	..*	..
	200 ,, ..	..*	..*	..	..*	..*	..
	400 ,, ..	..*	..*	..	..*	..*	..
Zinc	50 ,, ..	100	8·85	Normal	100	8·75	Normal
	100 ,, ..	100	8·31	do.	100	8·01	do.
	200 ,, ..	100	7·85	do.	100	7·75	do.
	400 ,, ..	100	7·69	do.	100	7·32	do.
Control I (Soaked in distilled water)		100	5·21	do.	100	5·00	do.
Control II (Unsoaked) ..		100	4·95	do.	100	4·95	do.

* No germination.

TABLE IV

Virulence of Fusarium vasinfectum *in Micro-Element Amended "Wilt-Sick" Soil*

Micro-elements	Levels in p.p.m.	No. of seeds sown	Percentage germination	Percentage pre-emergence wilt	Percentage post-emergence wilt
Aluminium	50	25	100	Nil	10
	100	25	100	,,	Nil
	200	25	100	,,	,,
	400	25	100	,,	,,
Boron	50	25	80	10	20
	100	25	40	Nil	20
	200	25	4	,,	Nil
	400	25	Nil	..	,,
Cobalt	50	25	100	Nil	5
	100	25	100	,,	Nil
	200	25	100	,,	,,
	400	25	100	,,	,,
Lithium	50	25	100	,,	20
	100	25	100	,,	20
	200	25	100	,,	10
	400	25	100	,,	5
Manganese	50	25	80	20	60
	100	25	84	16	60
	200	25	88	12	40
	400	25	92	8	33
Molybdenum	50	25	100	Nil	36
	100	25	100	,,	32
	200	25	100	,,	12
	400	25	100	,,	Nil
Nickel	50	25	100	,,	12
	100	25	100	,,	4
	200	25	100	,,	4
	400	25	100	,,	Nil
Zinc	50	25	100	,,	4
	100	25	100	,,	Nil
	200	25	100	,,	,,
	400	25	100	,,	,,
Control (*a*) without inoculation	..	25	100	,,	,,
Control (*b*) with inoculation	..	25	88	12	36

extent while in the control 12% of the seeds were attacked by the cotton wilt pathogen.

Highest percentage of post-emergence wilt was observed in manganese treatments. At 50 and 100 p.p.m. the percentage wilt was 60, while in the control (*a*) it was only 36.

In aluminium and zinc treatments wilt was observed only at 50 p.p.m. level, the wilt percentage being 10 and 4 respectively.

Discussion

Experimental evidence by many workers has proved that the susceptibility of plants to diseases, particularly the attack of certain vascular pathogens, is conditioned to considerable extent by their nutrition. It seemed rather obvious that by combining *Fusarium* wilt resistance with soil amendments a condition might be established where *Fusarium vasinfectum* could be partially held in check by host resistance and the several complicating soil factors (Pincard and Leonard, 1944).

The above results viewed in the light of the earlier experiments indicate the choice of zinc in preference to the other micro-elements tried. Zinc, as has already been shown, considerably suppressed the saprophytic potentialities of *Fusarium* spp., by retarding the colonising ability, by bringing about an early extermination of the pathogen from infected stubbles and by increasing the numbers of soil microflora as well as inhibiting spore germination in soil *in situ*. Further, the cotton plants grown in zinc amended soils showed significant increase in green and dry matter. Recent pot-scale experiments by Sarojini (1951) and Yogeswari (1950) clearly evidenced the efficacy of zinc in the effective control of ' gram-wilt ' caused by *F. udum* and ' foot-rot ' of paddy caused by *F. moniliforme*.

Although any one factor may decide disease incidence that of nutrition, as related to host, pathogen and related microflora remains basic throughout. The results presented herein, though not of great value until after confirmation with large-scale field trials, are nevertheless of interest in indicating a fresh line of attack of the wilt problem.

Summary

In the experiment conducted to study the response of cotton plants to micro-element soil amendments zinc and manganese were found to be beneficial for the growth of cotton plants. Boron, lithium and molybdenum were toxic.

Cent per cent. germination of cotton seeds was recorded in the control and all the treated soils except in the case of boron treatments. When the seeds were soaked in micro-element solutions prior to sowing in soil, only the nickel-treated seeds showed scanty germination.

Studies on the pathogenic potentialities of *Fusarium vasinfectum* on a susceptible variety of cotton showed that zinc was effective in reducing the wilt incidence, whereas manganese aggravated the incidence of wilt.

ACKNOWLEDGEMENTS

The author is greatly indebted to Professor T. S. Sadasivan for his helpful criticism and constant guidance in the course of this investigation. She thanks the University of Madras for the award of a studentship during the tenure of which this investigation was undertaken.

REFERENCES

Brenchley, W. E. and Warrington, K. "The role of boron in the growth of plants," *Ann. Bot.*, 1927, **41**, 167–87.

Padwick, G. W. .. "The genus *Fusarium*, III. A critical study of the fungus causing wilt of gram (*Cicer arietinum* L.) and of related species in the sub-section Orthocera with special relation to variability of key characteristics," Report of the Imperial Mycologist, *Sci. Rep. Agric. Res. Inst.*, New Delhi, 1940, **10**, 863–73.

Pincard, J. A. and Leonard, O. A. "Influence of certain soil amendments on the yield of cotton affected by the *Fusarium—Heterodera* complex," *J. Amer. Soc. Agron.*, 1944, **36**, 829–43.

Sarojini, T. S. .. "Soil Conditions and Root Diseases, II. *Fusarium udum* Disease of Red Gram [*Cajanus cajan* (Linn.) Millsp.]," *Proc. Indian Acad. Sci.*, 1951, **33**, 49–68.

Yogeswari, L. .. "Trace element nutrition of fungi with special reference to soil *Fusarium* spp." (Unpublished). Thesis approved for the Degree of Doctor of Philosophy, University of Madras, 1950.

MORPHOLOGICAL AND OTHER STUDIES ON TWO *NOCARDIA* SPECIES

By J. R. Vakil, S. R. Khambata and J. V. Bhat
(*Microbiology Department, St. Xavier's College, Bombay*)

Received November 26, 1952
(Communicated by Prof. P. Maheshwari, F.A.SC.)

Introduction

Despite the fact that the study of the actinomycetes has resulted in the accumulation of a voluminous mass of literature, it is only during the last decade and a half that workers like Henrici, *et al.* (1947), Waksman (1950) and others have succeeded in bringing order out of the chaotic state of knowledge regarding the morphology and taxonomy of this important group of micro-organisms which forms a sort of a connecting link between bacteria and fungi. In recent years the discovery of the antibiotics streptomycin, aureomycin and chloromycetin has given a great impetus to the study of actinomycetes. But at the same time it must be pointed out that attention has been paid chiefly to the genus *Streptomyces* which has so far been the most fruitful source in the hunt for newer antibiotics. Likewise, most of the cytological studies have been centred on *Streptomyces*. Only very recently has the genus *Nocardia* been given appreciable cytological attention by McClung (1950) and Morris (1951). The genus *Nocardia*, like the other genera of the order *Actinomycetales*, displays extreme plasticity in morphology. The student, on encountering such forms, soon finds himself entangled in a bewildering maze until careful, systematic and repeated observations have familiarised him with the peculiarities of this highly pleomorphic genus. It is hoped that the present paper dealing with some studies carried out on *Nocardia corallina* and *Nocardia rubra* isolated from two different sources will be of some use to those unfamiliar with *Nocardia*.

Experimental

The two *Nocardia* species, *viz.*, *N. corallina* and *N. rubra*, were isolated from coconut oil on glycerol agar as well as from the alimentary canal of the common Indian earthworm on Ashby's nitrogen-free agar. *N. corallina* was isolated from the gizzard while *N. rubra* was obtained from the œsophagus. Details regarding the technique of isolation have been described elsewhere (Vakil, 1950; Khambata, 1952).

V 36 1952 XUM

Morphological Studies

On Nutrient Agar.—Cultures were grown on nutrient agar and gram stained smears were examined microscopically at intervals of 24, 48 and 72 hours of incubation at room temperature (27° C.). After 24 hours, *N. corallina* showed gram positive, large, curved, filamentous and mostly " Y "-shaped branching rods (Plate XIII, Fig. 1). The strain obtained from coconut oil however showed few branched forms. Within 2 days the branched rods fragmented into short cylindrical rods, an important characteristic, so typical of the genus *Nocardia*. After 3 days, short, somewhat squarish coccoid forms were observed (Plate XIII, Fig. 2) indicating thereby almost complete fragmentation of the branched mycelium. The other *Nocardia* isolate, *N. rubra*, showed after 24 hours, gram positive fllaments with occasionally branched " Y "-shaped rods which were very short when compared to *N. corallina* (see Plate XIII, Fig. 3). These fragmented into short rods and coccoid cells within 3 days (Plate XIV, Fig. 4). The coccoid forms of both *Nocardia* species when subcultured on fresh media, germinated into mycelia, thus completing the life-cycle.

Acid-fast staining was also carried out on cultures grown on nutrient agar. The strains isolated from the earthworm were non-acid-fast while only *N. rubra* isolated from coconut oil, revealed weak acid-fastness.

Motility of all the cultures was, of course, negative.

On Ashby's Nitrogen-free Mannitol Agar.—The occasion for utilising Ashby's medium—a medium unsuitable for the study of actinomycetes—would not have arisen were it not for the fact that one of us (Khambata, 1952) had isolated, from the body of the earthworm, these micro-organisms on Ashby's agar. A spectacular change in morphology was observed on this medium. It was therefore desirable that besides the *Nocardia* species isolated from the earthworm, even those from coconut oil, be studied on Ashby's medium. On this medium, it was found that the strains of *N. corallina*, though isolated from two different sources, behaved in an identical manner. So did also the *N. rubra* strains.

As has already been mentioned, the morphology exhibited on Ashby's agar showed a remarkable change from that observed on nutrient agar. After 24 hours, *N. corallina* displayed unevenly stained, slender, curved, branched filaments, which gave a barred appearance (Plate XIV, Fig. 5). Within 3 days the filaments fragmented into short, plump, bulging, oval rods which showed characteristic deeply stained bipolar granules with an almost unstained vacuolated centre (Plate XIV, Fig. 6). These granules were also seen when smears were stained with ordinary methylene blue.

Due to this peculiar bipolar staining they almost look like the plague bacillus —*Pasteurella pestis*. The bipolar staining effect was also observed when the organism was grown on Conn's glycerol asparaginate agar (Plate XV, Fig. 9).

N. rubra, after 24 hours showed gram positive, slender, unevenly stained rods (Plate XV, Fig. 7) which fragmented within 2 days to form short rods which showed characteristic deeply stained granules situated subcentrally and not at the poles as in the case of *N. corallina* (Plate XV, Fig. 8).

Such bipolar granulation on nitrogen-free media has also been reported by McClung (1950) in the case of *Proactinomyces ruber* (now termed *Nocardia rubra*). Without detracting from the merits of McClung's observations, though he was the first to report on this change in morphology, it may be pointed out that our observations on this aspect of the morphology of the *Nocardia* were made independently of McClung's and before we could gain access to his paper.

It was further observed by us that these granules could also be stained by Neisser's, Albert's and Pugh's stains, commonly employed for the staining of volutin granules in corynebacteria. This strongly suggested the volutinic nature of these granules. A few microchemical tests were carried out on 2 to 3 days old cultures grown on Ashby's nitrogen-free agar in order to find out whether or not these granules possessed the solubility properties of volutin. Strangely enough, they were found to be insoluble in water at 80° C. for 10 minutes or in a 0·02 per cent. sodium bicarbonate solution for 2 hours. Evidently, the granules did not possess the solubilities of volutin. McClung (1950) also obtained similar results. He studied them in great detail with the help of the electron microscope and a number of microchemical tests. He found that the granules did not possess the solubilities of volutin but those of nuclear nucleoproteins. However, he could not detect the presence of thymonucleic acid by the Feulgen reaction. He concluded that on the basis of microchemical tests no definite role could be ascribed to these granules.

Cultural Characters

(*a*) *N. corallina*.—On nutrient agar, after 3 days, light orange pigmented colonies with filamentous margin. Colour of the growth after 8 days was Rufous, Pl. XIV 9′. OR–O.b (Ridgway, 1912). After 30 days the pigment deepened to Coral Red, Pl. XIII 5′. OO–R.b (Ridgway).

On Ashby's mannitol agar, moderate growth of minute yellowish orange slightly convex colonies with filamentous to arborescent margin.

V36 1952 XUM

On potato, after 8 days, colour of the growth was Rufous.

In nutrient broth, slight pellicle, fairly heavy turbidity and loose fibrous sediment.

(*b*) *N. rubra.*—On nutrient agar, after 3 days, pink colonies with filamentous margin. After 8 days, colour of the growth was Coral Pink, Pl. XIII 5′. OO–R.d (Ridgway).

On Ashby's mannitol agar, moderate growth of minute pinkish orange colonies with arborescent margin.

On potato, after 8 days, the colour of the growth was Carrot Red, Pl. XIV 7′. R–O.b (Ridgway).

In nutrient broth, slight pellicle, moderate turbidity and flocculent sediment.

Physiological Characters

(*a*) *N. corallina.*—Indole not produced; hydrogen sulphide produced; nitrate reduced to nitrite; alkalinity with orange ring and sediment in litmus milk; gelatin not liquefied; glucose and glycerol fermented with acid. The earthworm strain fermented mannitol also. The strain from coconut oil was tested for lipolytic action and casein hydrolysis. Both tests were recorded as negative.

The difference in the utilisation of paraffin, phenol and *o*-cresol by the two different strains can be seen in Table I.

TABLE I

Strain	Utilisation of		
	Paraffin	Phenol	*o*-Cresol
Earthworm strain ..	++	+*	–
Coconut oil strain ..	++++	++++	–

Legend: ++++ = Excellent growth.
++ = Fairly good growth.
+* = Poor growth after 7 days.
– = No growth.

(*b*) *N. rubra.*—Indole not produced; hydrogen sulphide produced; nitrate reduced to nitrite; alkalinity with orange ring and sediment in litmus

milk, gelatin not liquefied; lactose, glucose, saccharose, maltose, mannitol, glycerol, dextrin and starch not fermented. The strain from coconut oil gave negative results when tested for lipolysis and casein hydrolysis. Table II shows the differences in the utilisation of paraffin, phenol and *o*-cresol by the two different strains.

TABLE II

Strain	Utilisation of		
	Paraffin	Phenol	*o*-Cresol
Earthworm strain ..	++	+++	–
Coconut oil strain ..	++++	++++	–

Legend: ++++ = Excellent growth.
+++ = Good growth.
++ = Fairly good growth.
– = No growth.

DISCUSSION

The foregoing account reveals that *Nocardia* species undergo phenomenal pleomorphic changes during their life-cycle on various culture media. To establish the identity of an actinomycete as belonging to the genus *Nocardia*, it is imperative that a detailed study of its morphology be made. Although cultural and physiological studies do, to a certain extent, help towards the identification of species, it would be worthwhile studying the nutritional requirements of these micro-organisms, and as Pridham and Gottlieb (1948) have done, to use these as an aid to species determination. Furthermore, it would also be interesting to study the morphology of various species of *Nocardia* on Ashby's nitrogen-free agar and to establish the nature and significance of the peculiar deeply staining cytoplasmic granules which were observed by us. It is our earnest desire to carry out further work and to report on a future occasion both on the cytology and the nutrition of *Nocardia*.

SUMMARY

The morphological, cultural and physiological characters of strains of *Nocardia corallina* and *Nocardia rubra* isolated from coconut oil and the alimentary canal of the earthworm are described. Deeply staining granules in the cytoplasm of the *Nocardia* when grown on Ashby's nitrogen-free

medium have been reported. *N. corallina* showed bipolar granules while *N. rubra* showed subcentrally situated granules. The observations are substantiated with photomicrographic illustrations. The need for further study of the cytology and nutrition of the *Nocardia* is emphasised.

ACKNOWLEDGMENT

The authors gratefully acknowledge the kind assistance given by Dr. H. R. Guard, M.D., Pathologist to the Parsi General Hospital, Bombay, for providing the photomicrographic illustrations.

LITERATURE CITED

Henrici, A. T., Skinner, C. E., Emmons, C. W. and Tsuchiya, H. M. .. *Molds, Yeasts and Actinomycetes*, 2nd Ed., John Wiley & Sons, Inc., New York, 1947.

Khambata, S. R. .. "A Contribution to the Study of the Bacterial Flora Associated with the Intestine of the Earthworm as Revealed by the Enrichment Culture Method." M. Sc. Thesis, Bombay University, 1952.

McClung, N. M. .. "Morphological Studies in the Genus *Nocardia*," *J. Bact.*, 1950, **59**, 589–602.

Morris, E. O. .. "Observations on the Life-Cycle of the *Nocardia*," *J. Hyg.*, 1951, **49**, 175–80.

Pridham, T. G. and Gottlieb, D. .. "The Utilization of Carbon Compounds by Some *Actinomycetales* as an Aid for Species Determination," *J. Bact.*, 1948, **56**, 107–14.

Ridgway, R. .. *Color Standard and Color Nomenclature*, Published by the author, Washington, D.C., 1912.

Vakil, J. R. .. "Studies on the Production of Rancidity in Coconut Oil," M.Sc. Thesis, Bombay University, 1950.

Waksman, S. A. .. *The Actinomycetes*, The Chronica Botanica Co., Waltham, Mass., 1950.

EXPLANATION OF PLATES

FIGS. 1 & 2. Photomicrographs of *Nocardia corallina* on nutrient agar. (1) 24 hours, gram stain, × 900. (2) 72 hours, gram stain, × 900.

FIGS. 3 & 4. Photomicrographs of *Nocardia rubra* on nutrient agar. (3) 24 hours, gram stain, × 900. (4) 72 hours, gram stain, × 900.

FIGS. 5 & 6. Photomicrographs of *Nocardia corallina* on Ashby's nitrogen-free mannitol agar. (5) 24 hours, gram stain, × 900. (6) 72 hours, gram stain, × 900 (3 times enlarged).

FIGS. 7 & 8. Photomicrographs of *Nocardia rubra* on Ashby's nitrogen-free mannitol agar. (7) 24 hours, gram stain, × 900. (8) 48 hours, gram stain, × 900 (2 times enlarged).

FIG. 9. Photomicrograph of *Nocardia corallina* on Conn's glycerol asparaginate agar, 48 hours, gram stain, × 900 (4 times enlarged).

FIG. 1

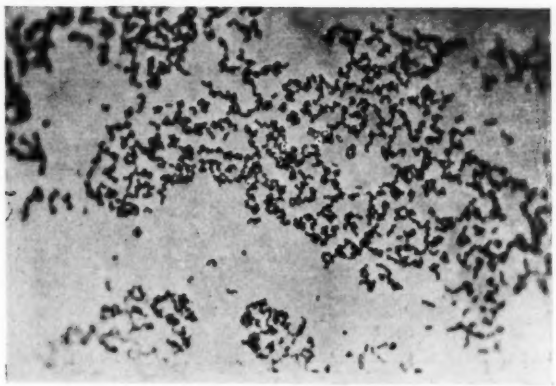


FIG. 2

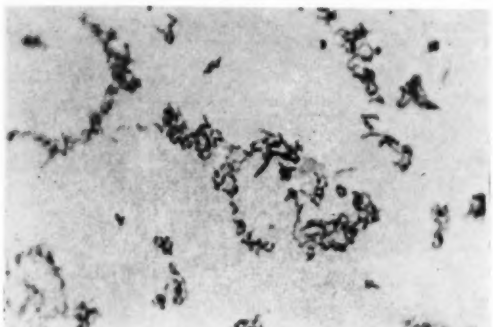


FIG. 3

V36 1952 XUM

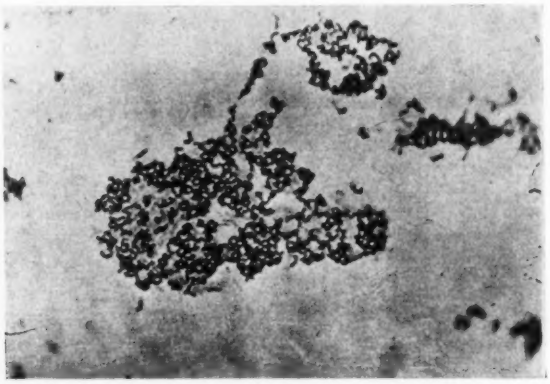


FIG. 4

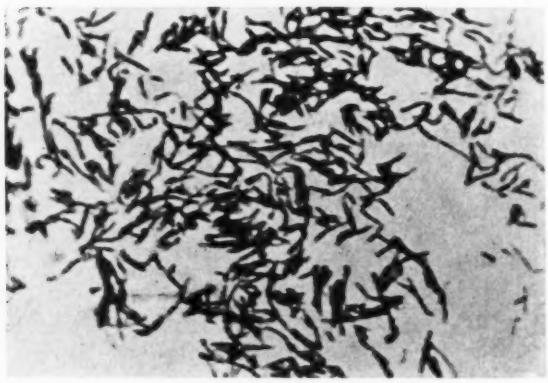


FIG. 5

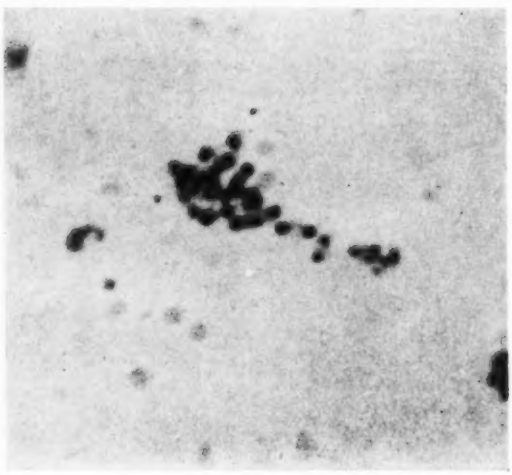


FIG. 6

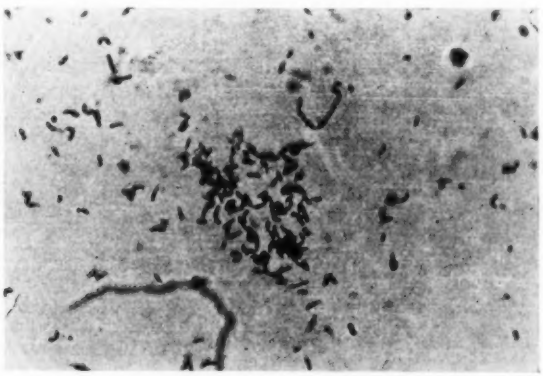


FIG. 7

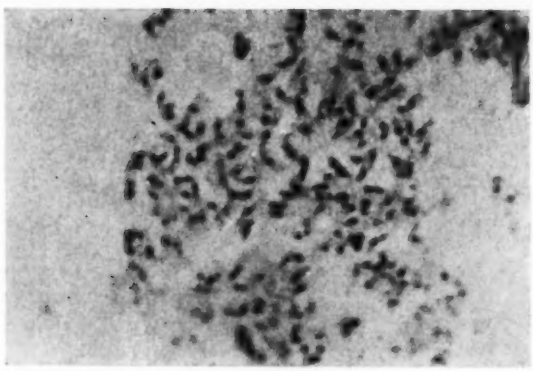


FIG. 8

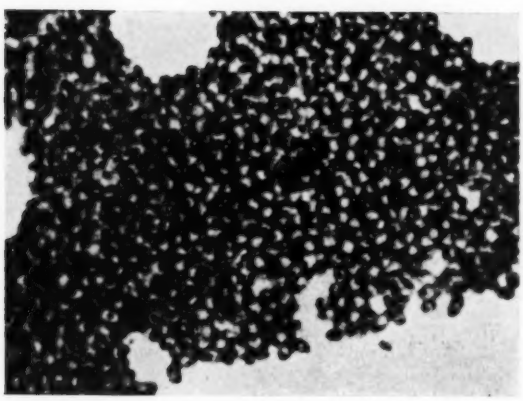


FIG. 9

V36 1952 XUM

EMBRYOLOGY OF THE DIPSACACEÆ

I. Glands, The Male and Female Gametophytes

BY B. A. RAZI AND K. SUBRAMANYAM

(Central College, Bangalore)

Received September 26, 1952

(Communicated by Dr. L. Narayana Rao, F.A.SC.)

INTRODUCTION

THE structure of the Dipsacalean flower and its vasculation have been ably expounded by Szabo (1923). Schnarf (1931) has reviewed the previous embryological literature on the family. Souèges (1937) has described the embryogeny of *Scabiosa succissa*, and Poddubnaja-Arnoldi (1933) has given an account of the male gametophyte in *Scabiosa purpurea*. More recently Philipson (1947) has dealt with the development of the inflorescence in *Scabiosa pratense* and *Dipsacus fullonum*. The present paper deals with the development of the glands and some aspects of the male and female gametophytes in *Dipsacus leschenaultii*, *Scabiosa caucasica* and *Cephalaria ambrosioides*.

MATERIALS AND METHODS

Material of *Dipsacus leschenaultii* collected in Kodaikanal on September 28, 1933, and preserved in formalin-acetic-alcohol, was available in the Department of Botany, Central College, Bangalore. *Scabiosa caucasica* and *Cephalaria ambrosioides* were collected in the Brooklyn Botanic Garden on June 24, 1946, by Prof. P. Maheshwari, and these were also preserved in formalin-acetic-alcohol. Some more material of *Scabiosa* was kindly sent by Mr. L. G. Nickell of the Brooklyn Botanic Garden during June-July, 1950. Customary methods of dehydration and imbedding were followed; sections were cut at a thickness of 10 to 16 microns and stained in Heidenhain's iron-alum hæmatoxylin or safranin-light green. The second method gave better results. A few whole mounts of the ovule were made in lactophenol in order to study the vascular supply of the ovule.

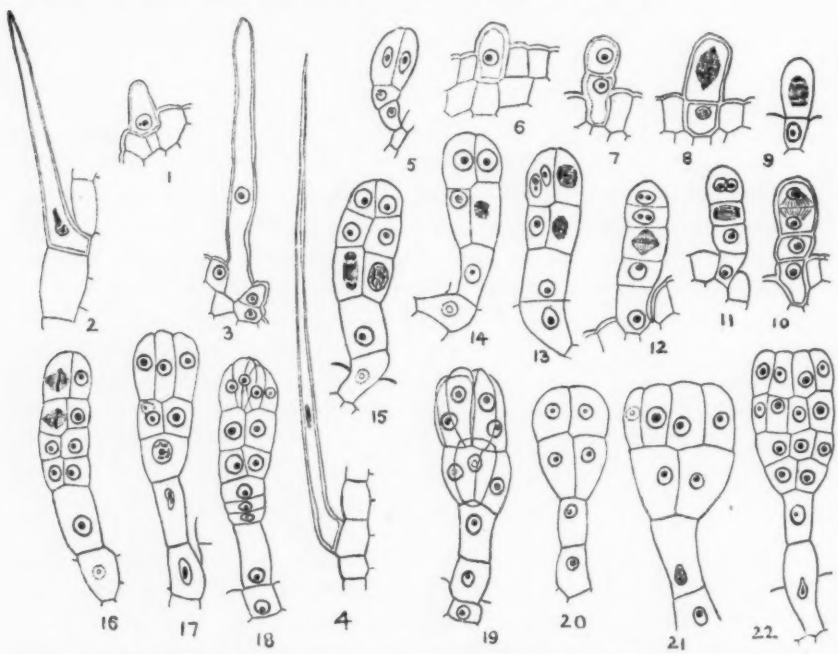
HAIRS AND GLANDS

Unicellular hairs are seen on the outer wall of the inferior ovary, disc, calyx and corolla. In the young hair the nucleus is situated at the base (Figs. 1, 2); but it soon migrates upwards (Figs. 3, 4). The hairs on the calyx and corolla are long and taper to a fine point (Figs. 2, 4) whereas those on the disc are more or less blunt (Figs. 1, 3). The hairs occurring on the

basal part of the corolla are directed downwards, while those in the upper part point upwards.

Multicellular glands occur on the calyx, corolla and disc. All these are directed upwards in the flower. Each gland consists of a multicellular head borne upon a uniseriate stalk made up of a variable number of cells. The filamentous stalk is usually straight, but the stalk of the glands on the calyx is slightly curved (Fig. 5).

Developmental stages of the glands on the disc and calyx have been studied. To start with, an epidermal cell elongates (Fig. 6) and protrudes out in the form of a papillate initial. It then divides transversely forming an outer and an inner cell (Fig. 7). The inner cell gets merged in the epidermal layer (Figs. 7 to 13). The outer cell by further transverse divisions (Figs. 8 to 10 and 12) produces a uniseriate row of a variable number of cells. At this stage vertical walls are laid down in the upper cells (Figs. 11, 13 to 15) resulting in a biseriate row. Further vertical divisions (Figs. 13 to 16) result in the formation of a multicellular gland (Figs. 17 to 22). The remaining cells in the lower region constitute the stalk (Figs. 16 to 22).



FIGS. 1–22. *Cephalaria ambrosioides*.—Figs. 1–4. Unicellular hairs. 1–3 × 200; 4, × 190. Figs. 5–22. Developmental stages of glands. All × 200 except 22, × 190.

The Inflorescence

All the species studied possess flowers in globose capitula. Each capitulum is subtended by a whorl of tough bracts which completely cover the young buds. The individual flowers are bracteate, zygomorphic and epigynous, with a very prominent disc capping the ovary.

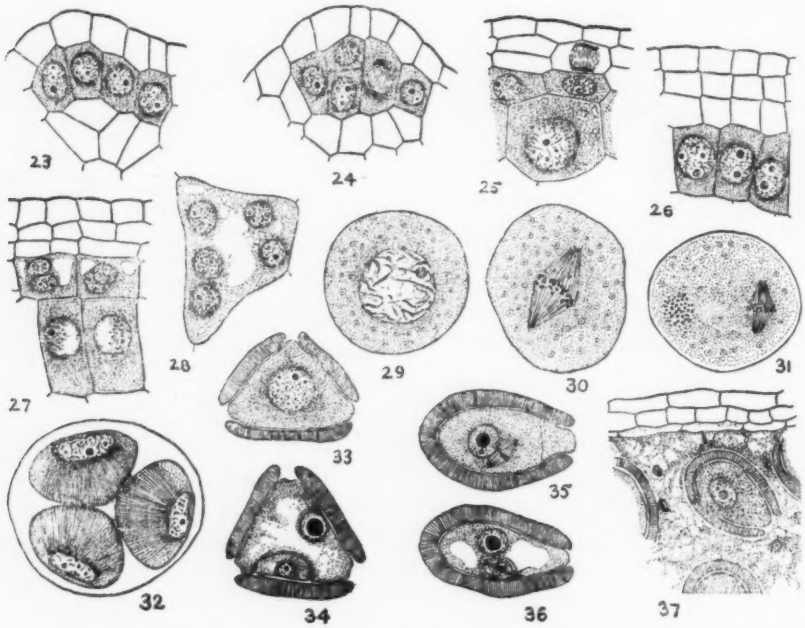
The inflorescence in *Cephalaria* has a convex axis on which the flowers are arranged. The outermost series of flowers possess a flattened ovary due to lack of space on the axis. In such flowers the ovule is initiated in the form of a nucellar primordium (Fig. 38) but does not develop further; no trace of even the archesporium has been seen in any preparation made from such flowers. Thus, the outermost series of flowers is seen to be functionally male while the rest of the flowers in the inflorescence are hermaphrodite. This feature has not been noticed so far in *Scabiosa* and *Dipsacus*.

Microsporangium and the Male Gametophyte

A transverse section of the young anther lobe shows a plate of four to five hypodermal archesporial cells (Fig. 23) which divide periclinally (Fig. 24) to form the primary parietal and primary sporogenous cells. The primary parietal layer undergoes further periclinal divisions to form the anther wall (Figs. 25 to 27), and the sporogenous cells after undergoing a few more divisions become converted into spore mother cells. The outermost layer in the anther is the epidermis, next comes the endothecium, then a middle layer and finally the tapetum (Fig. 27). The tapetum is uninucleate to start with (Fig. 25) but later becomes multinucleate (Figs. 27, 28). At a later stage during the development of the male gametophyte the tapetal walls disorganise resulting in a periplasmodium (Fig. 37). Schnarf (1931) quotes Juel as stating that the anther tapetum of *Knautia sylvatica* becomes amœboid without completely dissolving, that similar observations have been made by Strasburger on *Scabiosa caucasica* and *Cephalaria tartarica*; and that Risse has noted an amœboid anther tapetum in *Knautia arvensis*. Schnarf also says that, in contrast to the observations of Juel, Strasburger and Risse, there is found a disintegrated cytoplasm between the pollen grains of *Succisella inflexa*, *Scabiosa prolifera*, *Cephalaria leucantha*, *Pterocephalus brousonetii* and *Dipsacus silvester*.

The microspore mother cells undergo the usual reduction divisions (Figs. 29 to 31) and form tetrads of microspores. Quadripartition of the microspore mother cells takes place by peripheral cleavage furrows, and the microspores are arranged tetrahedrally (Fig. 32). The uninucleate microspore has a thick exine and a thin intine (Fig. 33). As a result of the first division

of the microspore a small lenticular generative cell (Fig. 34) is cut off from a large tube cell. The generative cell divides (Fig. 35) to produce two male cells. This is the shedding stage of the pollen grain (Fig. 36) which is tricolpate. The presence of two male cells has also been reported for *Scabiosa purpurea* (Poddubnaja-Arnoldi, 1933).

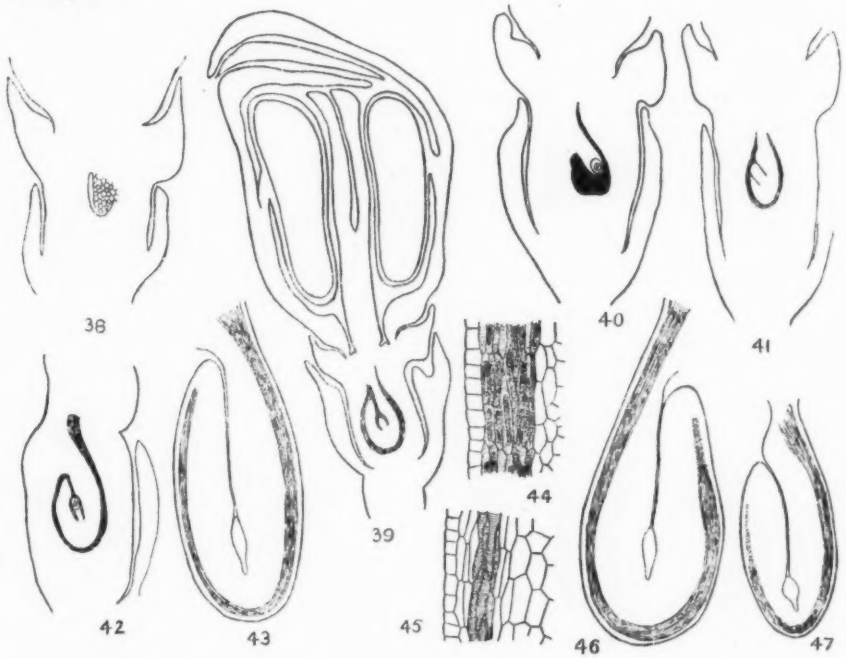


FIGS. 23–37. *Scabiosa caucasica*.—Fig. 23. Part of cross section of anther showing archesporial cells, × 243. Fig. 24. Division of archesporial cells to form the primary parietal layer and the primary sporogenous layer, × 243. Figs. 25–27. Stages in development of anther wall, × 243. Fig. 28. Multinucleate tapetal cell, × 243. Fig. 29. Microspore mother cell. Note starch grains, × 435. Figs. 30–32. Stages in the formation of microspore tetrad, × 435. Fig. 33. Uninucleate microspore, × 435. Fig. 34. Tube nucleus and lenticular generative cell, × 146. Fig. 35. Division of generative nucleus, × 146. Fig. 36. Mature pollen grain showing tube nucleus and male cells, × 146. Fig. 37. Tapetal periplasmodium, × 100.

MEGASPORANGIUM AND THE FEMALE GAMETOPHYTE

The ovary has a single loculus bearing a pendulous anatropous ovule (Fig. 39) which is tenuinucellate and has a single massive integument. It arises as a mass of cells from the top of the loculus and grows down into the cavity (Fig. 40). Soon there is a curvature (Fig. 41) resulting in the typical anatropous condition (Fig. 42). By the time the archesporium is

differentiated the single integument completely surrounds it. The micropyle is very long and narrow. There is a short and thick funiculus in the form of a pad.

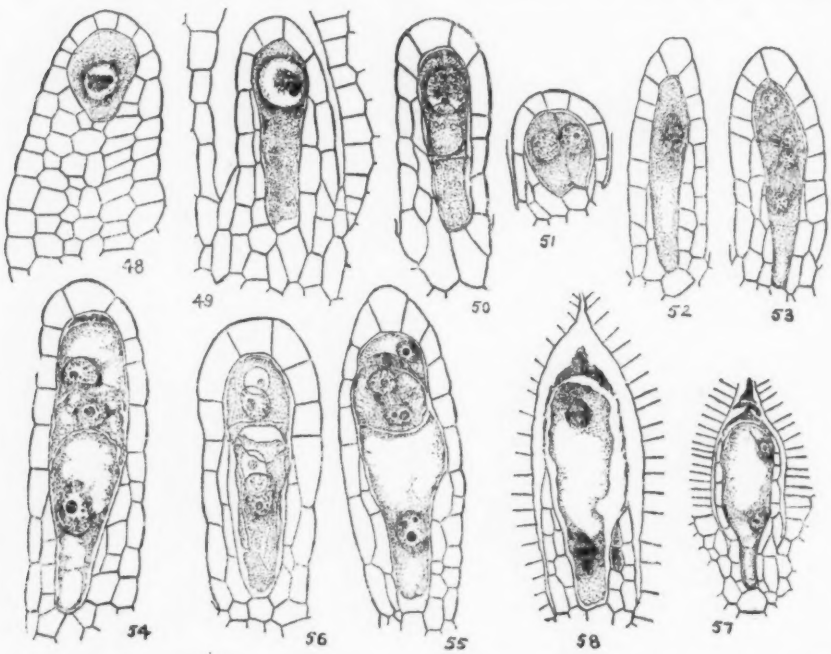


FIGS. 38–47. Figs. 38–45. *Cephalaria ambrosioides*; Fig. 46. *Dipsacus leschenaultii* and Fig. 47. *Scabiosa caucasica*. Fig. 38. L.S. ovary to show initiation of ovule in loculus, × 25. Fig. 39. L.S. flower bud, × 12. Figs. 40–42. Stages in development of anatropous ovule, × 25. Fig. 43. L.S. ovule to show vascular traces, × 25. Fig. 44. Part of the ovular integument to show elongation of sub-epidermal cells before vascularisation, × 146. Fig. 45. Same after cells develop spiral thickenings, × 146. Fig. 46. Outline of ovule and vasculature, × 15. Fig. 47. L.S. of ovule to show vasculature, × 15.

The vascular supply to the ovule arises at the funiculus and follows the outline of the ovule right up to the micropylar end (Fig. 43). The massive integument consists of several layers of rectangular isodiametric cells. Some of these cells lying below the outer epidermis become elongated and are disposed in the direction of the longitudinal axis of the ovule (Fig. 44). These elongated cells are densely cytoplasmic and meristematic. A few of these later transform themselves into vascular elements and develop the characteristic thickenings of the xylem (Fig. 45). This transformation of meristematic cells into xylem elements is seen to occur right up to the micropylar end (Fig. 43) in the ovule of *Cephalaria*; to proceed half way up around

the integument (Fig. 46) in *Dipsacus*; and to stop (Fig. 47) near the basal part of the ovule in *Scabiosa*. Such a tendency for the extension of the vascular strand into the integuments has also been noted in other members of this family (Szabo, 1923; Lavialle, 1925).

There is a single celled archesporium (Fig. 48) which functions directly as the megaspore mother cell (Fig. 49). Sometimes two megaspore mother

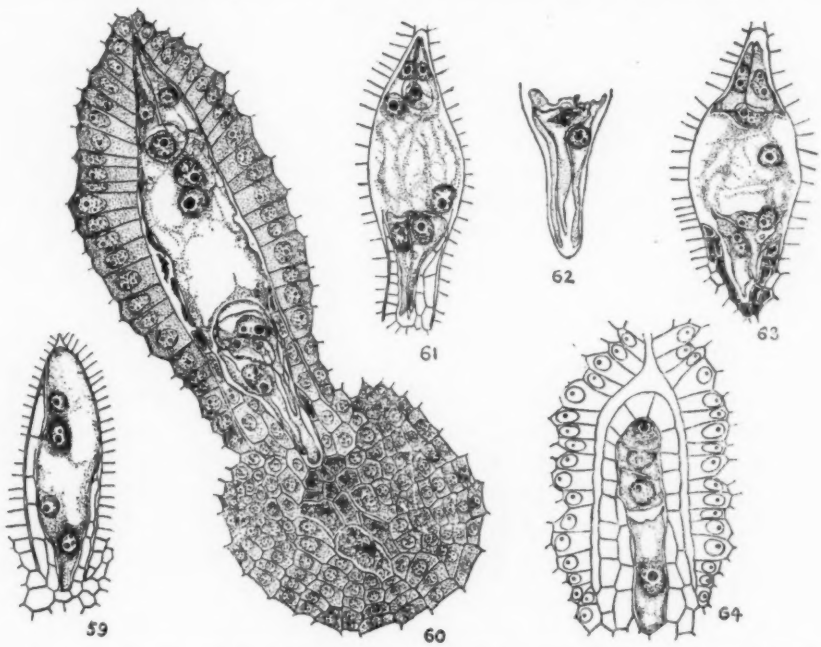


FIGS. 48–58. *Cephalaria ambrosioides*.—Fig. 48. Archesporium, × 243. Fig. 49. Megaspore-mother cell, × 243. Fig. 50. Double megaspore mother cells, × 240. Fig. 51. Double megaspore mother cells of *Scabiosa*, × 243. Fig. 52. Megaspore mother cell of *Dipsacus* dividing, × 243. Fig. 53. Dyads of *Dipsacus* dividing, × 243. Fig. 54. Linear tetrad of megaspores in which the uppermost has degenerated and the lowermost has elongated, × 146. Fig. 55. Linear tetrad of megaspores in which the third megaspore has formed a two nucleate embryo sac, × 243. Fig. 56. Double dyad in a superposed position, × 243. Fig. 57. Two nucleate embryo sac, × 108. Fig. 58. Formation of two nucleate to four nucleate embryo sac, × 108.

cells may be present in the same ovule in *Cephalaria* (Fig. 50) and *Scabiosa* (Fig. 51). The megaspore mother cell has a broad micropylar and a tapering chalazal end (Fig. 49), which abuts on a group of cells whose contents are seen to have collapsed. Beyond these cells the nucellar tissue is arranged in a characteristic radiating manner, which persists upto the mature embryo

sac stage (Fig. 60). This tissue has been regarded as the hypostase in *Knautia* (Lavialle, 1925). In *Knautia* it consists of a group of small, thin-walled cells, in which a number of schizogenous cavities are formed. These branch and anastamose and become filled with a yellowish substance, which also spreads into the antipodal cells and other adjacent tissue.

Two divisions in the megaspore mother cell (Figs. 52, 53) result in a linear tetrad of megaspores. An instance of a double dyad, one superposed



FIGS. 59–64. Figs. 59, 60 & 62. *Cephalaria ambrosioides* ; Fig. 63. *Scabiosa caucasica* ; Figs. 61 & 64. *Dipsacus leschenaultii*.—Fig. 59. Four nucleate embryo sac, × 108. Fig. 60. Mature embryo sac ; note prominent endothelium, collapsed cells at lower end of embryo sac, and radially arranged cells, × 435. Fig. 61. Mature embryo sac, × 146. Fig. 62. Antipodal cells ; note division of nucleus in one of them, × 243. Fig. 63. Mature embryo sac, × 146. Fig. 64. Linear tetrad of megaspores, note endothelium, × 243.

over the other, has been noticed in *Cephalaria* (Fig. 56). In *Cephalaria* sometimes the third megaspore of the linear tetrad develops further (Fig. 55) producing a two nucleate embryo sac. The chalazal megaspore is the functional one (Figs. 54, 55, 64) and after undergoing three more divisions (Figs. 57, 58, 59) produces an eight nucleate embryo sac of the Polygonum type (Figs. 60 to 63).

The mature embryo sac is spindle shaped and tapers at both ends. The embryo sac of *Dipsacus* (Fig. 61) is broader in the middle than that of *Cephalaria* (Fig. 60), and it is broadest in *Scabiosa* (Fig. 63). The egg apparatus consists of a pair of synergids with a large pear-shaped egg in between. Usually the two polars meet in the upper portion of the embryo sac very near the egg apparatus. The three antipodals are organised as definite cells (Figs. 60 to 63) and their lower ends taper into the narrow chalazal end of the embryo sac. Their structure, position and behaviour shows that they are probably haustorial in the case of *Cephalaria*; further, in this form their nuclei are seen to divide once (Fig. 62) so that each antipodal cell becomes binucleate. They have not been seen to divide in *Dipsacus* and *Scabiosa*, and in the latter they degenerate.

The mature embryo sac is invested with a prominent endothelium (Fig. 60). It consists of transversely elongated cells with dense contents and prominent nuclei in which two to three nucleoli are commonly seen. During development of the embryo sac, the nucellar epidermis is destroyed, and the innermost layer of the integument now comes into direct contact with the embryo sac and thus becomes modified into the endothelium, which is recognisable even at the dyad stage in *Cephalaria* and at the tetrad stage in *Dipsacus* (Fig. 64).

SUMMARY

A comparative account of some aspects of the embryology in three members of the Dipsacaceæ, *viz.*, *Dipsacus leschenaultii*, *Scabiosa caucasica* and *Cephalaria ambrosioides* is presented. In all the three species the flowers are borne on globose capitula. In *Cephalaria* the outermost flowers in the capitula are functionally male, since the ovule does not develop beyond the initial stages.

The wall of the anther is made up of the epidermis, endothecium, middle layer and tapetum. The cells of the tapetum soon become multinucleate and at a later stage form a periplasmodium. The mature pollen grain is tricolpate with a tube nucleus and two male cells.

In the pendulous tenuinucellate ovule the vascular strand traverses the single massive integument right up to the micropylar end of the ovule. The hypodermal archesporium directly functions as the megaspore mother cell. Double megaspore mother cells are found in *Scabiosa* and *Cephalaria*, and double dyads are seen in the latter. Megasporogenesis proceeds normally and the development of the embryo sac follows the Polygonum type.

The antipodals are organised as cells and in *Cephalaria* they become binucleate.

Surrounding the narrow chalazal end of the embryo sac there is a prominent radiating tissue.

Our sincere thanks are due to Prof. P. Maheshwari for kindly passing on to us the material used in this study, and also for going through the manuscript; to Mr. L. G. Nickell for collecting some of the material and Prof. L. N. Rao for encouragement.

Literature Cited

Lavialle, P. .. "Sur la nutrition du sac embryonnaire chez *Knautia arvensis*," *C. R. Acad. Sci.*, Paris, 1925, **180**, 2055–56.

Philipson, W. R. .. "Studies in the development of the inflorescence. II. The capitula of *Succissa pratensis* Moench., and *Dipsacus fullonum* L.," *Ann. Bot.* N. S., 1947, **11**, 285–99.

Poddubnaja-Arnoldi, P. .. "Spermazellen in der Familie Dipsacaceæ," *Planta*, 1933, **21**, 381–86.

Schnarf, K. .. *Vergleichende Embryologie der Angiospermen*, Berlin, 1931.

Souèges, R. .. "Embryogénie des Dipsacées. Développement de l'embryon chez le *Scabiosa succissa* L.," *C. R. Acad. Sci.*, Paris, 1937, **204**, 292–94.

Szabo, Z. .. "The development of the flower of Dipsacaceæ," *Ann. Bot.*, 1923, **37**, 325–34.

1140-62 Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent, and Published by The Indian Academy of Sciences, Bangalore

INDEX TO VOL. XXXVI (B)

AUTHORS' INDEX

Ayyappa, P. K. and Cheema, P. S.	An ectoparasite on the larvæ of *Anthrenus vorax* Waterhouse, 215.
Bhat, J. V. ..	*See* Vakil and others.
Bhimachar, B. S. and George, P. C.	Observations on the food and feeding of the Indian Mackerel, *Rastrelliger canagurta* (Cuvier), 105.
Cheema, P. S. ..	*See* Ayyappa and Cheema.
Desai, S. V. and Subbiah, B. V.	Relative value of nitrogen and phosphate in some organic manures and inorganic fertilisers and their combinations as judged by their recovery by crops, 181.
Dnyansagar, V. R. ..	Embryological studies in the leguminosæ, IV, 1.
George, P. C. ..	*See* Bhimachar and George.
Kalyanasundaram, R. ..	*See* Satyanarayana and Kalyanasundaram.
	Ascorbic acid and *fusarium* wilted plants, 102.
Khambata, S. R. ..	*See* Vakil and others.
Murthy, A. R. Vasudeva	*See* Rao and others.
Narayanan, E. S. and Venkatraman, T. V.	Technique of mass multiplication of *Stenobracon deesæ* (Cam.) hymenoptera: braconidæ for use against sugarcane and maize borers, 12.
Pai, K. V. Srinivasa ..	*See* Venkata Ram and Pai.
Prakash, Ravi and Saksena, R. K.	Decomposition of paddy and Bajra *Pennisetum typhoideum* straws by fungi commonly found in Allahabad soils, 119.
Raju, M. V. S. ..	Embryology of *Anagallis pumila* Swartz., 34.
Ramakrishnan, T. S. ..	Observations on ergots on *Pennisetum* and other grasses, 97.
Ramakrishnan, T. S., Srinivasan, K. V. and Sundaram, N. V.	Additions to the fungi of Madras, XIII, 85.
Rao, A. Nagaraja ..	*See* Subramanyam and Rao.
Rao, B. Sanjiva, Murthy, A. R. Vasudeva, and Subrahmanya, R. S.	The amylose and the amylopectin contents of rice and their influence on the cooking quality of the cereal, 70.
Razi, B. A. and Subramanyam, K.	Embryology of the dipsacaceæ, I, 249.

Saksena, R. K. .. *See* Prakash and Saksena.

Samuel, Mary .. A new species of cœlomic trematode of the genus *Staphylorchis* from the tiger shark *Galeocerdo tigrinus* from Indian waters, 169.

Satyanarayana, G. and Kalyanasundaram, R. Soil conditions and root diseases, V, 54.

Shanti Devi .. Studies in the order parietales, III, 59.

Solomon, S. .. Studies in the physiology of phanerogamic parasitism with special reference to *Striga lutea* Lour. and *S. densiflora* Benth. on *Andropogon sorghum* Hack, II, 198.

Srinivasan, K. V. .. *See* Ramakrishnan and others.

Subbiah, B. V. .. *See* Desai and Subbiah.

Subrahmanya, R. S. .. *See* Rao and others.

Subramanian, C. V. .. Fungi imperfecti from Madras—I, II, III, 43, 160, 223.

Subramanyam, K. .. *See* Razi and Subramanyam.

Subramanyam, K. and Rao, A. Nagaraja A contribution to the embryology of *Chlorophytum allenuatum* Bak., 191.

Sulochana, C. B. .. Soil conditions and root diseases, IV, 19, VI, 229, VII, 234.

Sundaram, N. V. .. *See* Ramakrishnan and others.

Tampi, P. R. S. .. *See* Viswanathan and Tampi.

Vakil, J. R., Khambata, S. R. and Bhat, J. V. Morphological and other studies on two *Nocardia* species, 243.

Venkatakrishniah, N. S. .. *Glomerella psidii* (Del.) Sheld. and *Pestalotia psidii* Pat., associated with a cankerous disease of guava, 129.

Venkata Ram, C. S. and Pai, K. V. Srinivasa Fertilization value of tank silts,, II, 81.

Venkatraman, T. V. .. *See* Narayanan and Venkatraman.

Vimal, K. P. .. Spores and pollen from tertiary lignites from Dandot, West Punjab (Pakistan), 135.

Viswanathan, R. and Tampi, P. R. S. .. Oxygen consumption and viability of *Chanos chanos* (Forskål) in relation to size, 148.

TITLE INDEX

Amylose and the amylopectin contents of rice and their influence on the cooking quality of the cereal (Rao and others), 70.

Anagallis pumila Swartz., embryology (Raju), 34.

Ascorbic acid and *fusarium* wilted plants (Kalyanasundaram), 102.

Chlorophytum attenuatum Bak., a contribution to the embryology (Subramanyam and Rao), 191.

Cœlomic trematode of the genus *Staphylorchis*, a new species, from the tiger shark *Galeocerdo tigrinus* from Indian waters (Samuel), 169.

Dipsacaceæ, embryology, I (Razi and Subramanyam), 249.

Ectoparasite on the larvæ of *Anthrenus vorax* Waterhouse (Ayyappa and Cheema), 215.

Ergots on *Pennisetum* and other grasses, observations (Ramakrishnan), 97.

Fungi imperfecti from Madras, I, II, III (Subramanian), 43, 160, 223.

Fungi of Madras, additions, XIII (Ramakrishnan and others), 85.

Glomerella psidii (Del.) Sheld. and *Pestalotia psidii* Pat., associated with a cankerous disease of guava (Venkatakrishniah), 129.

Leguminosæ, embryological studies, IV (Dnyansagar), 1.

Nitrogen and Phosphate, relative value, in some organic manures and inorganic fertilisers and their combinations as judged by their recovery by crops (Desai and Subbiah), 181.

Nocardia species, two, morphological and other studies (Vakil and others), 243.

Order parietales, studies, III (Shanti Devi), 59.

Oxygen consumption and viability of *Chanos chanos* (Forskål) in relation to size (Viswanathan and Tampi), 148.

Paddy and Bajra, *Pennisetum typhoideum*, straws, decomposition, by fungi commonly found in Allahabad soils (Prakash and Saksena), 119.

Phanerogamic parasitism, studies in the physiology, with special reference to *Striga lutea* Lour. and *S. densiflora* Benth. on *Andropogon sorghum* Hack, II (Solomon), 198.

Rastrelliger canagurta (Cuvier), Indian Mackerel, food and feeding, observations (Bhimachar and George), 105.

Soil conditions and root diseases, IV (Sulochana), 19, V (Satyanarayana and Kalyanasundaram), 54, VI, VII (Sulochana), 229, 234.

Spores and pollen from tertiary lignites from Dandot, West Punjab (Pakistan) (Vimal), 135.

Stenobracon deesæ (Cam.) Hymenoptera, mass multiplication, technique: braconidæ for use against sugarcane and maize borers (Narayanan and Venkatraman), 12.

Tank silts, fertilization value, II (Venkata Ram and Pai), 81.